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Canadian Journal of Research

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INFLUENCE OF THE AMINO ACID – DEXTROSE REACTION ON GROWTH OF *BACILLUS POLYMYXA*[1]

BY RUTH PETERSON, DYSON ROSE, AND L. LOEB

Abstract

Growth of *B. polymyxa* over a 72 hr. period was not significantly affected by the brown, fluorescent materials formed by the reaction between dextrose and protein constituents of the media. During the first 24 hr. after inoculation a slight inhibition was evident in autoclaved yeast extract media, and in casein hydrolyzate media to which additional amino acid – dextrose reaction products had been added, but the inhibitory effects were never marked and disappeared within 48 hr.

Introduction

During studies on the *Bacillus polymyxa* fermentation (2, 4) it was noted that heating the media resulted in a partial inhibition of the growth of this organism (4, 5) and this inhibition was attributed to the brown, fluorescent products formed by the reaction between reducing sugars and protein constituents of the media. Recent studies showed that products of this reaction did not inhibit growth of lactobacilli (6) and it was therefore of interest to test the assumption that these products inhibited *B. polymyxa*.

Materials and Methods

A pure strain (N.R.C. No. C42) of *B. polymyxa* was maintained on agar slants and transferred to 10 ml. of liquid medium 24 hr. before inoculation of the experimental media. One drop of this culture was used to inoculate 10-ml. aliquots of experimental media. Cultures were maintained at 26.7° C.

This organism produces a mucoid substance that made turbidity measurements of little value for following growth. Growth was therefore followed by determining the amount of carbon dioxide produced by cultures grown in Eldredge tubes containing 5 ml. of medium and 5 ml. of 3% dextrose solution in one arm and 10 ml. of standard (approx. 0.3 *N*) barium hydroxide in the other. The barium hydroxide was added to the tubes just prior to inoculation; immediately afterwards the tubes were corked and sealed with paraffin. At the end of 24-, 48-, and 72-hr. growth periods, two tubes were shaken gently for 15 min. to facilitate carbon dioxide adsorption and the residual hydroxide was

[1] *Manuscript received July 16, 1949.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as paper No. 75 on the Industrial Utilization of Wastes and Surpluses, and as N.R.C. No. 2020.

[The October issue of Section C (Can. J. Research, C, 27: 191-268. 1949.) was issued November 7, 1949.]

titrated with standard hydrochloric acid. Control tubes containing uninoculated media were included and the carbon dioxide produced by the organism was calculated from the difference in titration between control and culture tubes. Nonproliferating cells can, of course, produce carbon dioxide, but under the conditions used in these studies the organism developed rapidly in all cultures and it seems probable that carbon dioxide production was an adequate measure of growth.

The constituents of the yeast extract and of the casein hydrolyzate media are shown in Table I. Good growth of this organism occurs over a fairly wide

TABLE I

COMPOSITION OF THE MEDIA

Constituents	Casein hydrolyzate medium	Yeast extract medium
Yeast extract (Difco)	—	1.25 gm.
Casein hydrolyzate (Smaco)	12.5 ml.	—
Vitamin solution (3)	5.0 ml.	—
Sodium phosphate, monobasic	100 mgm.	12.5 mgm.
Sodium phosphate, dibasic	100 mgm.	12.5 mgm.
Sodium chloride	20 mgm.	2.25 mgm.
Calcium chloride	22.5 mgm.	2.5 mgm.
Zinc sulphate	2.25 mgm.	0.25 mgm.
Sodium hydroxide	To pH 7.0	To pH 7.0
Total volume	250 ml.	250 ml.

pH range (1, 4), and preliminary tests, under the conditions used in these experiments, indicated that autoclaving media of pH 7.0 did not change the pH sufficiently to affect growth of the organism.

The relative concentration of the amino acid – dextrose reaction products was estimated by determining the fluorescence of uninoculated cultures (6) using a Coleman Model 12 Photofluorometer and B-1 and PC-1 filters. The units reported are those of the instrument scale, the photofluorometer being adjusted to give a scale reading of 50.0 for a solution containing 0.200γ of quinine sulphate per milliliter.

Results

Influence of the Sterilization Procedure

Carbon dioxide production by the organism was determined in media sterilized by autoclaving, at 121° C. for 13 to 15 min. with dextrose present, in media to which sterile dextrose solution was added after autoclaving, and in media sterilized by filtration through a type ST, size L6 Seitz pad. Three trials were conducted with each medium, duplicate tubes being titrated at each sampling time.

Casein hydrolyzate media had an average fluorescence of 18 to 19 when filtered or when autoclaved without dextrose. Autoclaving with dextrose increased the fluorescence to 30, 48, and 32.5 in the three trials, the variation presumably being due to minor differences in autoclaving technique.

Yeast extract contains brown, fluorescent materials and media containing it had higher fluorescence readings (44 to 46 for filtered material) than casein hydrolyzate media. Traces of reducing sugars in yeast extract eliminate much of the advantage otherwise gained by autoclaving the main supply of dextrose separately; the fluorescence of media treated in this manner was 58 to 60. Media autoclaved with all the dextrose present had a fluorescence of 66 to 68.

Fig. 1 shows the average carbon dioxide production with each medium. Only average results are given except where the differences exceeded the 5% level of statistical significance. Thus, carbon dioxide production in casein

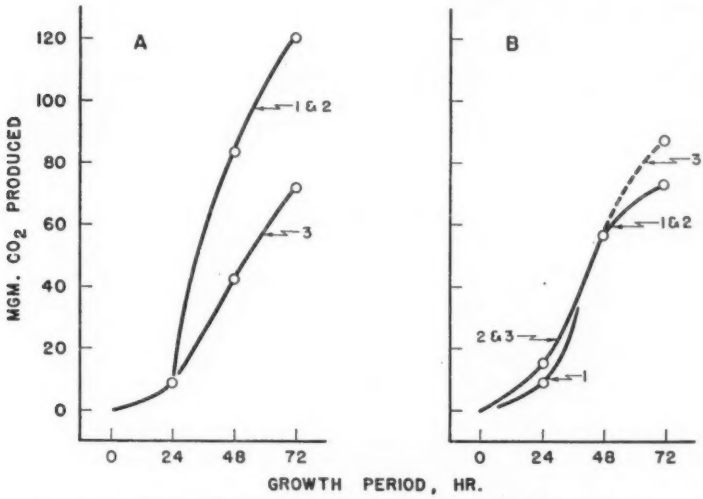

FIG. 1. *Effect of method of preparation of media on growth of B. polymyxa. A—casein hydrolyzate media; B—yeast extract media.*

Curve 1—Medium autoclaved with dextrose.
Curve 2—Dextrose autoclaved separately.
Curve 3—Filtered medium.

hydrolyzate media (Fig. 1, A), autoclaved with and without dextrose, did not differ significantly and only one line is shown. On the other hand, the amount of carbon dioxide produced on filtered media was significantly lower than on autoclaved media at the 48 and 72 hr. periods.

Results on yeast extract media were more reproducible than those on casein hydrolyzate. Carbon dioxide production in the first 24 hr. was significantly lower on media autoclaved with dextrose (Fig. 1, B) than on the other two

media but at the 48 hr. point this difference had disappeared. At 72 hr., growth on the filtered media significantly exceeded that on the autoclaved media.

Influence of Added Amino Acid – Dextrose Reaction Products

Soluble amino acid – dextrose reaction products were obtained by subjecting a mixture of casein hydrolyzate and dextrose to a temperature of 60° C. for several days. The brown solution thus obtained was filtered and suitable aliquots were mixed with 2 gm. of dextrose and made to 100 ml. These solutions were sterilized and 5 ml. added aseptically to tubes containing 5 ml. of autoclaved, dextrose-free casein hydrolyzate medium. The dextrose content of these tubes was thus 1% plus the residual dextrose of the brown solution, as compared with the 1.5% normally provided.

Triplicate trials were again made using control tubes (av. fluorescence 19) and two levels of added brown solution (av. fluorescence 91 and 203). Media at the highest fluorescence level had a transmission of about 15% of that of the controls, measured at 4200 Å.

Fig. 2 presents only the statistically significant results. At the end of the 24 hr. period, growth was inversely proportional to the fluorescence of the media, but at the 48 hr. point these differences had disappeared, and at 72 hr. the situation was reversed, greatest growth having occurred at the highest fluorescence level.

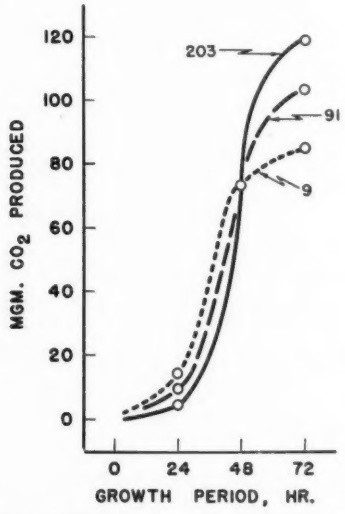

FIG. 2. *Effect of added fluorescent materials on growth of B. polymyxa.* (*Numbers indicate the relative fluorescence of the media.*)

Discussion

Although no direct experimental data are available, it appears probable that the Seitz filter pad adsorbed a nutrient from the casein hydrolyzate

medium and thus restricted growth of the organism. The superior growth at 72 hr. in filtered yeast extract medium may indicate a mild toxicity due to products formed during autoclaving, but the differences in fluorescence among these media are small.

Considerable unreacted dextrose remained in the solutions used in the experiments of Fig. 2, and the total carbon dioxide production in 72 hr. is undoubtedly a function of the available dextrose supply. The highest amount of fluorescent material present greatly exceeded the amounts developed by any normal autoclaving procedure; nevertheless, the organism was not only able to develop normally after the first few hours but was able to utilize the excess dextrose. It is quite evident, therefore, that any inhibition due to fluorescent materials was slight, and that the more serious inhibitions previously observed (4, 5) must have been due to some other factor such as destruction of a nutrient. This conclusion is essentially in agreement with that previously reached from studies on the lactic acid bacteria (6).

The results for the 24 hr. growth periods do indicate, however, that *B. polymyxa* is inhibited by the brown fluorescent materials to a slight extent during the early stages of growth. This is shown in both the media containing added fluorescent materials and in the yeast extract media, but small, transitory inhibitions are of little importance in fermentation studies.

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FUNDAMENTAL STUDIES ON A SALTANT OF *HELMINTHOSPORIUM SATIVUM*[1]

BY NORMAN JAMES,[2] N. E. R. CAMPBELL,[3] AND CHAIM GUNNER[4]

Abstract

A saltant of *H. sativum* disappeared from six different soils in which it was cultured in the laboratory within four weeks, whereas the indigenous flora persisted for nine weeks. During this nine week period numbers of bacteria showed no obvious trend, but numbers of fungi decreased. When cultured in sterilized portions of the above soils, the saltant showed a downward trend in numbers in the nine week period, a trend that was more obvious in some cultures than in others. Twenty-two of 221 isolates of fungi from soil, grain, and air, 45 of 286 isolates of *Streptomyces*, and 33 of 193 isolates of other bacteria produced evidence of antagonism to the saltant in spot inoculation studies. Filtrates prepared from two of the fungal isolates suppressed the growth of the saltant, as did also autoclaved portions of the filtrates. On the contrary, filtrates from three cultures of bacteria failed to suppress growth of the saltant, even though cultures of the bacteria did. The same 22 fungal isolates and 25 of the *Streptomyces*, when introduced separately into sterile soil along with the saltant, reduced the disease rating of barley seedlings below that produced by the saltant alone. The direct-count method of estimating numbers of spores of the saltant in a suspension was found to be reliable. Estimates based on the direct count were significantly greater than those made by the plate method. Estimates based on numbers of the saltant developing on potato dextrose agar containing small amounts of sterile soil were smaller than those made on the same medium without the soil. Even though the soil in the medium changed the pH of the medium, the difference in pH was considered not to be the primary factor in producing the lower estimates. Soil, either natural or heat sterilized, would contain the thermostable filterable substance or substances found to be produced by two species of fungi and probably produced by other species, likewise normally present in soil.

A saltant of *Helminthosporium sativum* P.K. & B., produced in 1932 by Greaney and Machacek (3), has retained the pathogenicity of the parent and remained relatively stable in cultural characteristics. However, when introduced into soil it loses its infectability. Field plots, heavily inoculated with the saltant, have produced a high incidence of disease only in the first crop (8). Since the saltant can be recognized readily on agar media, it was used in these studies to obtain information that might explain, in part at least, the varying incidence of the root rot disease of cereals (7).

Longevity in Soil Culture

The longevity of the saltant was determined by culturing in soil under controlled conditions and making plate counts at the beginning of the culture period and at intervals for nine weeks.

[1] *Manuscript received June 27, 1949.*

Contribution from the Department of Bacteriology and Animal Pathology, The University of Manitoba, Winnipeg, Man., with financial assistance from the National Research Council of Canada.

[2] *Professor of Bacteriology.*

[3] *Assistant Professor of Bacteriology.*

[4] *Graduate Assistant.*

Six samples of soils ranging in texture from sandy loam to clay (moisture equivalent from 14.8 to 56.8%) were air-dried at room temperature for several weeks. Then, enough of each sample was transferred to each of 20 50-ml. Erlenmeyer flasks to give 25 gm. of moisture-free soil. Each flask was fitted with a rubber stopper, through which was inserted a 2.5 mm.-bore glass tube plugged loosely with cotton. One half of the 20 replicates of each sample were sterilized at 15 lb. steam pressure for four hours on each of two consecutive days. The other half were used in the natural state. A suspension of conidia and fragments of mycelium of the saltant was prepared from Czapek's agar cultures by washing with sterile water and mixing thoroughly. Its density was checked by the Neubauer hemocytometer method. A 2 ml. aliquot of the suspension and sufficient sterile water were added to bring each sample to its moisture equivalent. One replicate of each of the natural and the sterilized samples was used for making the initial dilutions. From these, duplicate 0.001 dilutions were used in duplicate plates for the saltant and other fungi cultured in Czapek's agar medium at 25° C. for one week; and duplicate 0.000001 dilutions for bacteria cultured in sodium albuminate agar. Each estimate thus was based on counts on two plates from each of two dilutions. The other replicates were stored at 25° C. and used at weekly intervals for making estimates on the same basis as the previous estimates.

The saltant disappeared rapidly from all samples of natural soil. In no case was it found on any of the plates after the soil cultures had been stored for four weeks. This scarcely could be attributed to loss of moisture, since the greatest loss on any sample in the nine week period was 0.52%. The indigenous flora in the samples did not show the same trend during the period of storage. In all samples the numbers of fungi became less; whereas those of bacteria fluctuated, with no obvious trend. The results on one of the samples, presented in Table I, are typical.

TABLE I

SURVIVAL OF A SALTANT OF *H. sativum* AND OF OTHER FUNGI AND BACTERIA IN SOIL SAMPLE NO. 4; MOISTURE EQUIVALENT, 56.8%

Weeks	Natural soil			Sterilized soil
	Saltant $\times 10^3$	Fungi $\times 10^3$	Bacteria $\times 10^6$	Saltant $\times 10^3$
0	77.5	22.4	18.5	60.0
1	50.0	20.0	24.0	38.0
2	2.0	15.0	17.0	35.0
3	1.5	10.0	17.5	37.0
4	0.0	8.0	18.5	34.0
5	0.0	6.0	17.5	30.0
6	0.0	5.0	19.5	25.0
7	0.0	7.5	18.5	24.0
8	0.0	5.0	16.0	25.5
9	0.0	6.0	14.5	19.0

On the contrary, the saltant was isolated from all the sterilized samples on all dates, although in general, numbers showed a downward trend with time. These results are shown, along with those for raw or natural soil, in Fig. 1.

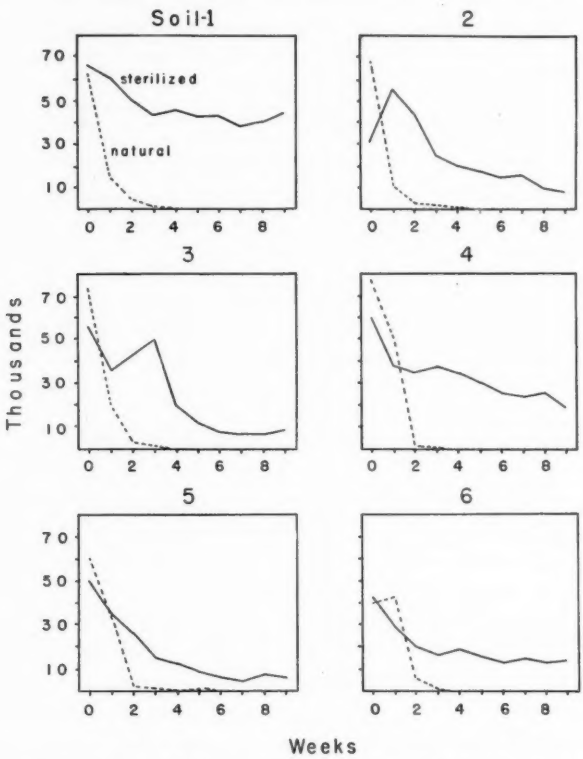


FIG. 1. *Survival of a saltant of H. sativum in raw and sterilized soils.*

Evidence of Antagonism in Plate Culture

Each of 221 isolates of fungi, picked at random from plates prepared from soil and flour and verified as to purity by replating, was tested for antagonism to the saltant by making spot inoculations of the isolate and the saltant 1 cm. apart on Czapek's agar previously allowed to harden in a Petri plate. Triplicate pairs of inoculations were made on one plate. Incubation was at 25° C. for about 10 days. Twenty-two of these isolates showed antagonism to the saltant by slowing its growth and causing its hyphae to be turned away, or in some cases by overgrowing it and suppressing its development.

Each of 286 isolates of the *Streptomyces* Waksman and Henrici was tested similarly by the spot inoculation procedure. Forty-five of these showed evidence of antagonism.

One hundred and ninety-three isolates of other bacteria were tested for antagonism to the saltant by a variation of the above method. A loopful of a 24 hr. broth culture of the bacteria was plated in nutrient agar. After the agar had hardened, three spot inoculations of the saltant were made to the surface of the medium. Incubation was at 25° C. for 10 days. Thirty-three of these produced evidence of antagonism by causing the growth of the saltant to be less than the normal on bacteria-free plates. In order to determine whether such a dwarfing effect on a fungus could be attributed to a filterable substance produced by a nutrient broth culture of bacteria, the following experiment was carried out. Three series of dilutions varying quantitatively in numbers of cells of *Bacterium herbicola* Burri and Duggeli and of *Pseudomonas* sp., two species found to be epiphytic on the seed of cereals (6), and of a mixed culture of the flora on a sample of wheat were tested against the saltant and against *Fusarium oxysporum* (Schlecht.) Snyder and Hansen by the spot inoculation method. A second set of plates containing filtrates prepared from nutrient broth cultures of these bacteria was tested against the two fungal strains. The procedure for testing the filtrates was as follows. Potato dextrose agar containing half of the normal amount of water was added to a series of plates with (*a*) 5 ml. sterile water, (*b*) 5 ml. of a filtrate from a 10 day culture of the bacteria, (*c*) 5 ml. of an 0.01 dilution of the filtrate, and (*d*) 5 ml. of an 0.0001 dilution of the filtrate, respectively. Then spot inoculations of each fungus were made as outlined above. With each culture of bacteria and with each strain of fungus the dwarfing effect was progressively less with dilution of bacteria (smaller inoculum), whereas there was no evidence of dwarfing on plates containing any concentration of the filtrates prepared from any of the bacterial cultures. A typical result is shown in Fig. 2. There was no evidence that these bacteria produced a water-soluble filterable substance antagonistic to the saltant or to *Fusarium oxysporum.*

The above result was not obtained when filtrates prepared from two species of fungi were tested against the saltant. Five ml. of a filtrate from a 10 day culture of *Aspergillus flavus* completely inhibited growth of the saltant; 5 ml. of a 0.2 dilution suppressed growth slightly; whereas 5 ml. of a 0.01 dilution had no effect. Heat sterilized portions of the filtrate produced essentially the same results. A filtrate of *Fusarium oxysporum* likewise was antagonistic to the saltant, although sterilizing appeared to lessen the antagonistic effect slightly. A typical result is shown in Fig. 3.

Reduction of Infection in the Presence of Antagonist

Only those isolates shown to be antagonistic to the saltant by the spot inoculation method were used for this study. The procedure was based on reports by Sallans (9) and Sanford and Cormack (10). For the first isolate, about 400 barley seeds were soaked in water for three hours, disinfected in

1 : 1000 mercuric chloride for seven minutes and rinsed five times in sterile water. One quarter of the seeds was transferred aseptically to a 60 ml. suspension of the saltant in sterile water; another quarter to a suspension of saltant and isolate; another to a suspension of the isolate; and the fourth to sterile water. The last two served as controls. After one hour the seeds were removed from the liquid (merely by inverting the bottle carefully) and incubated at 25° C. for 20 hr. Twenty seeds from each suspension were planted in each of two 1 liter flasks containing 300 gm. of a sterile, moist 3 : 1 soil–sand mixture. After 10 days, seedlings were rated for infection by the method of Greaney, Machacek, and Johnson (4). The other isolates were investigated in the same manner, except for the omission of the suspension of the saltant, on which the disease rating had been established. In every case seeds treated in the suspension of the isolate alone, and in sterile water, produced normal seedlings whereas those treated in a suspension of the saltant and a fungal isolate produced seedlings with a disease rating smaller than that produced by seeds treated in a suspension of the saltant alone. The results on the fungal isolates are shown in Table II. Likewise, 25 of the 45 isolates of the genus *Streptomyces* reduced the disease rating significantly below that of the saltant alone.

FIG. 2. *Effect of a filtrate from a portion of, and of a culture of Pseudomonas sp., on development of a saltant of H. sativum.*

Above—left to right:

Plate 1.—Saltant on double concentration potato dextrose agar containing 5 ml. sterile water.

Plate 2.—Saltant on the agar containing 5 ml. of an 0.0001 dilution of a filtrate from a 10-day culture of the bacteria.

Plate 3.—Saltant on the agar containing 5 ml. of an 0.01 dilution.

Plate 4.—Saltant on the agar containing 5 ml. of the filtrate.

Below—left to right:

Plate 1.—Same as Plate 1 above.

Plate 2.—Saltant on the agar freshly inoculated with 1 ml. of an 0.01 dilution of a culture of the bacteria.

Plate 3.—Saltant on the agar with 1 ml. of an 0.1 dilution.

Plate 4.—Saltant on the agar with 1 ml. of the broth culture.

FIG. 3. *Antibiotic effect of filtrate from 10 day culture of Aspergillus flavus on development of a saltant of H. sativum on double concentration potato dextrose agar.*

Above—natural filtrate of A. flavus cultured in potato dextrose liquid medium.

Below—sterilized filtrate.

Left to right:

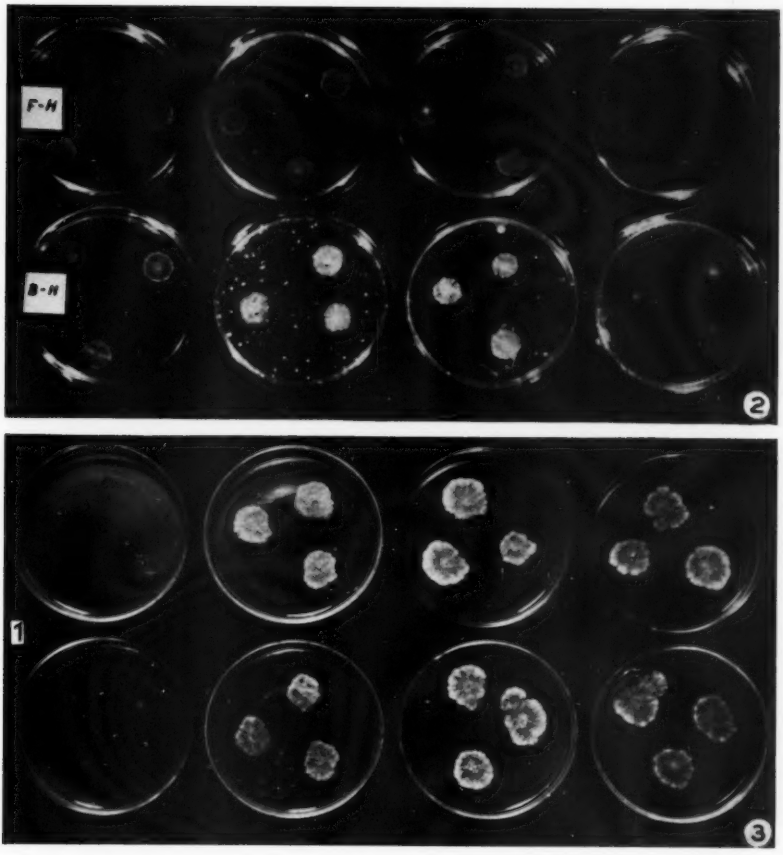
Plate 1.—Five ml. of filtrate.

Plate 2.—Five ml. of 0.2 dilution of filtrate.

Plate 3.—Five ml. of 0.01 dilution of filtrate.

Plate 4.—Five ml. of sterile water.

PLATE I



FIGS. 2-3.

TABLE II

EFFECT OF ISOLATES FOUND TO SHOW ANTAGONISM TO A SALTANT OF *H. sativum* IN PLATE CULTURE ON INFECTION OF BARLEY SEEDLINGS BY THE SALTANT

Isolate	Mean disease rating	
	Saltant and isolate	Difference from saltant*
Aspergillus candidus Link	23.5	−17.8
A. flavipes (Bain. and Sart.) Thom and Church	2.0	−39.3
A. flavus Link		
Isolate 1	1.0	−40.3
Isolate 2	10.5	−30.8
A. glaucus Link	20.0	−21.3
A. nidulans (Eidam) Winter	1.0	−40.3
Cladosporium herbarum (Persoon) Link	6.5	−30.8
Fusarium oxysporum (Schlecht.) Snyder and Hansen		
Isolate 1	1.0	−40.3
Isolate 2	1.0	−40.3
Mucor varians Povah	6.0	−35.3
Penicillium canescens Sopp	7.5	−33.8
P. cesiae Bainier and Sartory	3.5	−37.8
P. commune Thom	15.0	−26.3
P. corylophylum Dierckx		
Isolate 1	2.5	−38.8
Isolate 2	5.5	−35.8
P. hagemi Zaleski	18.0	−23.3
P. krzemieniewskii Zaleski	8.5	−32.8
P. lilacinum Thom	5.5	−35.8
P. nigricans Bainier-Thom	15.5	−25.8
P. pfefferianum (Wehmer) Westling	7.5	−33.8
P. purpurogenum Stoll	5.5	−35.8
P. roqueforti Thom		
Isolate 1	7.5	−33.8
Isolate 2	5.0	−36.3
P. roseo-maculatum Biourge	**	
P. steckii Zaleski	13.5	−27.8
P. terrestre Jensen	**	
Scopulariopsis brevicaulis (Sacc.) Bainier	1.0	−40.3
Trichoderma lignorum (Tode) Harz	3.0	−38.3

* *Necessary difference at 0.05 = 13.2 and at 0.01 = 17.5.*

** *Variance exceeded control limits established at the 3 sigma level (1).*

Estimating Numbers of the Saltant

While the principle of estimating fungal populations by the plate-count method is open to criticism, the fact holds that the method is used in relation to many different problems. It was used in this study for comparison with the direct-count method.

A Neubauer haemocytometer slide, employed in the routine quantitative determination of spores and fragments of mycelium of molds in certain foods, was used for the direct counts. The reliability (5) of an estimate based on this procedure was established, as follows. A suspension of spores and fragments of mycelium in sterile water was made from a 14 day Czapek's agar culture of the saltant. Counts of spores were made on five fields, each representing 0.0001 ml. of the suspension, and averaged. This was replicated

four times on one suspension. A Chi square value was calculated from the average counts on the four slides from one suspension, an additional degree of freedom being lost due to the use of average counts (2). This procedure was carried out on 96 different suspensions. The goodness-of-fit test (2) on the distribution of Chi square values obtained thus yielded a P value of 0.13. The results are shown in Fig. 4.

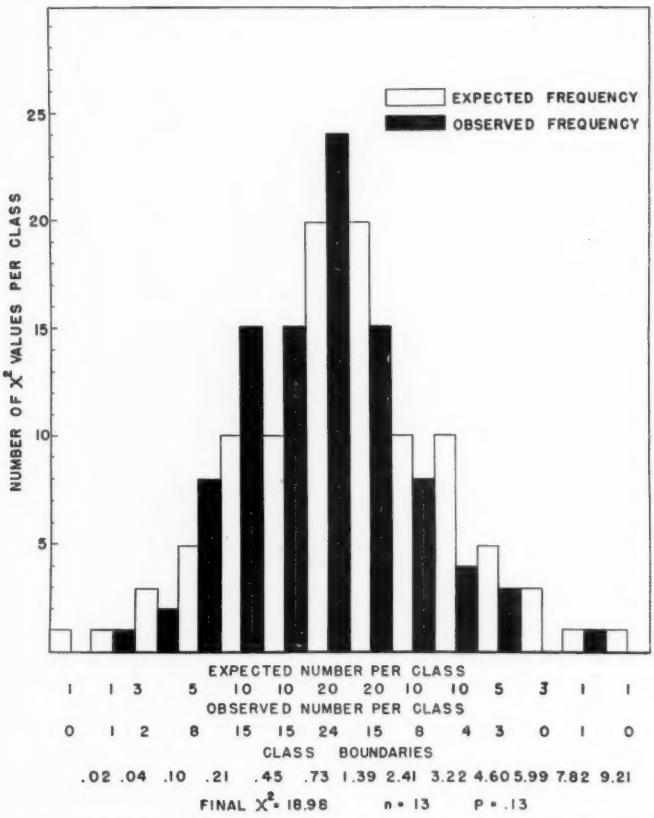

EXPECTED FREQUENCY
OBSERVED FREQUENCY
NUMBER OF X² VALUES PER CLASS
25
20
15
10
5
EXPECTED NUMBER PER CLASS
1 1 3 5 10 10 20 20 10 10 5 3 1 1
OBSERVED NUMBER PER CLASS
0 1 2 8 15 15 24 15 8 4 3 0 1 0
CLASS BOUNDARIES
.02 .04 .10 .21 .45 .73 1.39 2.41 3.22 4.60 5.99 7.82 9.21
FINAL X² = 18.98 n = 13 P = .13

FIG. 4. *Distribution of Chi square values calculated from direct counts of spores in 96 suspensions of the saltant.*

A study of the relationship between estimates made by the direct-count and the plate-count methods was carried out on a second set of 96 suspensions. Each estimate by the direct-count method was based on counts on five fields from each of four replicate slides prepared from one suspension and, by the plate-count method, on four replicate plates prepared from an 0.0002 dilution of the same suspension cultured in Czapek's agar, adjusted to pH 4.5, at

25° C. for seven days. Two estimates by the direct-count method were made on each suspension: (*a*) spores and (*b*) spores, plus fragments of mycelium. The latter estimates, obviously being larger on each suspension, were not considered further. Estimates by the plate-count method were smaller than those based on direct counts of spores on 92 of the 96 suspensions studied; and on the remaining four the difference was small. The difference between means of the 96 estimates made by the two methods was 80,340 per ml. The necessary difference (2) for significance at the 0.01 level was 44,320.

Effect of Soil in Medium on Estimates of the Saltant

The suspensions used in the studies referred to above were water suspensions. They lacked the soil particles and solutes that would be present when estimating a soil population. The following experiment was carried out to study the effect of sterile soil on estimates of the saltant based upon plate counts. Two gm. soil was sterilized with sufficient water to make the 90 ml. blank to be used for preparing the 0.0002 dilution of the suspension to be plated. The 1 ml. placed in each plate thus contained 0.02 gm. soil—or 100 times as much as would be present in plates prepared from soil at this dilution. The larger amount was used merely to make any effect more readily detectable. The experiment was replicated eight times on each of 12 different soils. It was carried out at the same time and with the same suspensions as were used in the experiment dealing with comparison of estimates by the direct-count and plate-count methods. The plate-count estimates on the water suspensions of the saltant thus served as the controls for this study.

In all of the 96 suspensions tested, estimates made from plates containing sterile soil were smaller than those from plates without soil. The analysis of variance follows:

Source	D.f.	Mean square	F	F at 0.05
Soils	11	63,332.27	50.32	1.90
Treatments × soils	12	9131.34	7.25	1.87
Replicates × soils	84	24,243.03	17.67	1.87
Error	84	1258.66		

The effect for the interaction of replicates and soils was expected, since the experiment was carried out with different cultures of the saltant and it was not feasible to prepare suspensions with the same number of spores.

Effect of Soil in Medium on pH of Medium

The following experiment was carried out to determine the effect of soil in the medium on pH of the medium. The pH of a batch of Czapek's medium

was determined. Then 0.02 gm. and 0.10 gm., respectively, of each of the 12 soils, sterilized in water suspension, were added to 10 ml. Czapek's liquid medium and the pH determined. The results, presented in Table III, show

TABLE III

EFFECT OF STERILE SOIL IN CZAPEK'S LIQUID MEDIUM ON pH OF THE MEDIUM

Soil	Control		Soil per 10 ml. medium			
			0.02		0.10	
	1	2	1	2	1	2
a	4.50	4.50	4.80	4.70	5.10	5.10
b			4.90	4.95	5.50	5.50
c			4.80	4.80	5.00	4.95
d			5.60	5.60	6.90	7.00
e			4.70	4.75	5.00	5.00
f			4.80	4.80	5.10	5.05
g			4.65	4.60	5.10	5.00
h			4.75	4.75	4.90	4.95
i			4.70	4.65	5.00	5.00
j			4.70	4.70	4.85	4.80
k			4.75	4.80	5.00	4.90

that with every soil the pH of the medium containing 0.02 gm. of soil was higher than that of the control and the pH of the medium containing 0.1 gm. soil was higher than that containing 0.02 gm. soil. It should be noted that the 0.02 gm. soil per 10 ml. was equivalent to the 0.02 gm. per plate used in the experiment reported immediately above. It is scarcely probable that the small difference in pH was the only factor responsible for the significantly lower estimates from plates containing soil in the medium.

Discussion

It appears obvious that the reduction in infection under field conditions was due to the destruction of this saltant in soil; and not merely to loss of infectability. In the laboratory experiments the destruction was greatest in natural soils—the saltant not being found after four weeks in any of the samples. But the effect was evident in heat sterilized soil—numbers of the saltant in sterilized soil showing a downward trend with time and numbers of the saltant that developed on Czapek's agar containing small amounts of soil being fewer than on the same medium without the soil. This would indicate that the destructive and inhibiting substance or substances probably was a product of the metabolism of the indigenous soil flora. It was produced in larger amounts in some soils than in others. Some was thermostable in soil. Even the relatively extreme sterilizing exposure did not inactivate all. The experiments on antagonism showed that it was produced by a number of isolates of fungi, *Streptomyces*, and bacteria (probably). It was filterable.

The spot inoculation method used for measuring antagonism showed a fair degree of agreement with the reduction-in-disease-rating method. However, correlation studies were not carried out since the data were not considered appropriate. The rating for antagonism was at three arbitrary levels only; whereas that for reduction of infection was at levels of 0.1 up to 41.3. The latter was the disease rating of the saltant in this study.

The finding that the bacterial isolates from wheat did not produce a filterable substance in sufficient quantity to be antagonistic to the saltant, or to *Fusarium oxysporum*, may be accepted as suggesting that the suppression of *Helminthosporium sativum* by the epiphytic bacteria on wheat seeds, reported by Simmonds (11), probably was due primarily to competition for nutrients in the medium. Preliminary incubation of moistened seed would produce a numerically increased active epiphytic flora, which would compete for nutrients in the medium surrounding the seeds. The effect would simulate that of antagonism.

Acknowledgments

The authors are pleased to express thanks to Dr. J. E. Machacek, Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, for his helpful interest in these studies and for checking the identity of the fungi listed, and to Associate Professor G. B. Oakland, Department of Actuarial Science of this university, for his advice on the statistical aspects of the investigations.

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A STUDY OF SUGAR BEET ROOTROT IN SOUTHERN ONTARIO[1]

By W. E. McKeen[2]

Abstract

In southern Ontario rootrot of sugar beets may be caused by *Aphanomyces cochlioides* Drechs., *Pythium aphanidermatum* (Edson) Fitz., *Pythium ultimum* Trow, or *Rhizoctonia solani* Kuehn. The distribution of these fungi was found to be limited to soil type rather than to locality, *A. cochlioides* thus being found in the clay soils and *P. aphanidermatum* only in the sand loam. They were not present in the subsoil and were most abundant in the top two or three inches of surface soil. *A. cochlioides*, which was found for the first time in Canada in 1946, causes the so-called blackroot of sugar beets, and is the most economically important pathogen encountered. Blackroot appears either as an early acute attack or a later chronic one, but the acute phase is of major importance and occurs in epiphytotics during seedling development, usually when the beet is from two to five weeks old. Weather records and experiments have shown that serious disease outbreaks only occur when moisture is abundant and soil temperature exceeds 60° F. Susceptibility is sharply correlated with the developmental rhythm of the seedling and ends when the cortex of the hypocotyl is sloughed off. *A. cochlioides* is homothallic, attacks the hypocotyl of the beet, and develops in the intercellular spaces of the cortex. It will not grow at relative humidities of less than 99% and this sensitivity to all but extremely high humidity is believed to limit its parasitic activity. Microbiological studies indicated that the number of organisms in the rhizosphere of diseased sugar beet roots is much larger than in that of healthy beet roots. Since parallel rhizosphere responses followed local artificial killing of roots, they are believed to be due in part at least to the utilization of necrotic tissues or substances released on their breakdown. Streptomycin, but not penicillin, was effective in controlling rootrot in greenhouse experiments. Control with Arasan is achieved in part through a direct fungicidal action, but the evidence suggests that it may be due to a shift in the microbiological balance of the soil flora unfavorable to the pathogen.

Introduction

In Ontario, sugar beets are grown most extensively in the southwestern part of the province, the largest acreages occurring in Kent, Lambton, Middlesex, and Elgin counties, with much smaller plantings in Huron, Perth, and Essex. Wh le most of the beets are produced on heavy Brookston and Clyde clay loam soils, some excellent crops are found on light sandy loam in the vicinity of Exeter in Huron, and tremendous yields are harvested from the marl soil of the Blackwell area in Lambton.

During the last 15 years, in the area indicated, about 27,000 acres of beets with an average yield of approximately nine tons per acre have been harvested annually. All of these beets have been marketed through the one channel, the Canada and Dominion Sugar Company, which has produced an average of 70,000,000 lb. of sugar, 11,100 tons of molasses, and 13,700 tons of dried beet pulp each year. In a single year the growers have received as much as

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Contribution No. 994, from the Department of Botany, University of Toronto, Toronto, Ont., and from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. Based on a thesis presented in April 1949, to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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four million dollars for the crop and, in addition, they have beet crowns and tops as green feed for livestock. The factory workmen, transportation agencies, and supply companies receive another two and a half million dollars annually so that, everything considered, the sugar beet industry has reached sizeable proportions in Ontario.

Although the sugar beet may suffer from many fungus, virus, and nematode diseases, most of these, up to the present time, have proved to be of little economic significance in Ontario. *Cercospora* leaf spot, which was once a devastating disease, has been completely controlled by the use of resistant varieties. At the present time rootrot is the most widespread and economically significant pathological condition with which the grower has to cope. It is primarily a seedling disease and is the major factor responsible for poor stands of beets. Faced with this uncertainty of obtaining and maintaining a good stand of plants and the possibility of losing an entire crop, the farmer is reluctant to plant his fields to beets, and an adequate acreage is difficult to maintain. The mechanization of the sugar beet industry, moreover, has made more urgent the need for controlling this disease, since the machines used for blocking and thinning operations operate on the basis of a practically perfect seedling stand.

In 1944 the Canada and Dominion Sugar Company reported that 685 acres, valued at approximately 90,000 dollars, were completely wiped out by rootrot and in many other fields various percentages of the seedlings were killed and very spotty stands resulted. In 1940 a still greater destruction was caused and every year the disease is present throughout the whole beet growing district. With continued production of beets it has tended to become more serious and, because of it, many growers no longer attempt to grow beets. In fact, rootrot was probably the major factor responsible for the abandoning of beet growing in Essex County.

The present study was undertaken to determine the identity and relative importance of the causal organisms responsible for root degeneration in sugar beets and to study the factors influencing the severity of such diseases as might be found in Ontario.

Survey of Pathogens Associated with Rootrot

To determine the organisms responsible for root decay, seedlings that showed the first symptoms of rootrot were removed from the soil and washed thoroughly in running water before they were placed in Petri plates containing agar media or water. Surface sterilization with mercuric chloride or alcohol was only necessary when the infected tissue was placed on the agar media. Most of the diseased seedlings from which isolations were made were obtained from commercial fields throughout the sugar beet district but additional isolations were made from sugar beet seedlings that had grown in infested soil in the greenhouse. The following fungi were obtained: *Aphanomyces cochlioides* Drechs., *Pythium aphanidermatum* (Edson) Fitz., *Pythium ultimum* Trow, *Rhizoctonia solani* Kuehn, *Fusarium* spp., *Penicillium* spp., *Actinomyces*

spp., *Mucor* spp., and some that were not identified. Frequently only one fungus emerged from the diseased seedling when it was placed in water. Pathogenicity tests were conducted by growing sugar beet seedlings in sterile soil inoculated with cornmeal and cultures of all the above organisms. In these tests only *A. cochlioides*, *P. aphanidermatum*, *P. ultimum*, and *R. solani* proved to be pathogenic.

The distribution of the pathogens was found to be related to soil type rather than to locality, as can be observed in Table I. *A. cochlioides* was

TABLE I

THE FUNGI ISOLATED AND THEIR RELATIVE FREQUENCY OF ISOLATION FROM SUGAR BEET SEEDLINGS GROWN IN FOUR SOIL TYPES CHARACTERISTIC OF THE AREAS OF COMMERCIAL BEET PRODUCTION

	Isolations				
	Total	Brookston clay	Clyde clay	Exeter sandy loam	Blackwell marl
A. cochlioides	164	137	27	0	0
P. ultimum	102	88	8	0	6
P. aphanidermatum	32	0	0	32	0
R. solani	18	15	2	0	1
Fusarium spp.	22	6	0	0	16
Penicillium spp.	5	3	0	2	0
Actinomyces spp.	6	4	2	0	0
Mucor spp.	7	5	0	1	1
Unidentified spp.	17	11	2	1	3

found only in the clay soils, whereas *P. aphanidermatum* was the only pathogen in the sandy loam of the Exeter district and was not found in any of the other soil types. *P. ultimum* and *R. solani* were present in all but the Exeter sandy loam. It was observed that in the Blackwell marl soil where rootrot was of no significance neither *A. cochlioides* nor *P. aphanidermatum* was present. Judging from the relative frequency of isolation and the disease symptoms observed in the field, *A. cochlioides* appeared to be the most virulent and economically important of the pathogens and for this reason was studied in some detail and is reported on first.

Blackroot Caused by *Aphanomyces cochlioides*

Symptoms

A. cochlioides, frequently designated as the water mold, causes what is commonly called blackroot of sugar beets, which is the most dreaded of the rootrot diseases. Blackroot appears either as an early acute attack or a later chronic one, but it is the acute phase that is of major importance and exhibited in epiphytotics during seedling development, usually when the beet is from two to five weeks old.

The symptoms in very young seedlings are not significantly different from those of seedlings attacked by *Pythium*, except that they assume a darker color, but those produced after true leaves are formed are striking and characteristic. A necrosis of the hypocotyl begins at the ground level, spreads rapidly to the cotyledonary leaves, and give the hypocotyl a characteristic shiny jet-black color that makes it appear as though scorched by a flame. This, no doubt, gave rise to the term blackroot, although this is somewhat of a misnomer and "black hypocotyl" would be more accurate.

In a short time the blackened cortex of the hypocotyl dries; the stem shrivels to a mere thread, and only the vascular system remains. The seedling topples over, withers, and is frequently broken by the wind. Fig. 1 shows seedlings with these characteristic symptoms. Whole fields of sugar beet seedlings may be destroyed within three or four days if the weather is moist and warm. If lower temperatures of around 50° F. prevail, infected seedlings may live for at least two weeks even in saturated soil. The leaves at first have a bluish tint and later slowly turn yellow as the fungus moves up the stem. If the soil dries and the temperature remains low, new lateral roots (Fig. 2) may be produced and the seedling recovers. The grower calls this 'coming out' of blackroot.

After the seedlings have produced three to five pairs of true leaves, the cortex of the hypocotyl fissures (Fig. 3) and this tissue is sloughed off owing to the formation of a periderm below. In the root the cortex drops off much earlier, usually when the seedling is about two or three weeks old. After the cortex is lost the seedling no longer suffers from the acute phase of attack.

In July and August the chronic phase of the disease may occur on the larger beets, the infected individuals usually being scattered sparsely throughout a 'blackroot' field. The affected plants show a wilting that becomes more severe from day to day and finally the leaves begin to die. When these plants are pulled out of the soil, infection will usually be found to have occurred in the extreme tip of the tap root and this necrotic tip is often embedded in wet soil. A slight amount of infection in this tip region may cause pronounced wilting. Three beets attacked in this manner are shown in Fig. 4. Very infrequently a beet may be invaded in the middle of the tap root (Fig. 5) in which case the lesion extends completely around the root and causes considerable constriction.

Isolation of Aphanomyces cochlioides

The generic name '*Aphanomyces*' means 'illusive fungus' and no better term could be applied to this pathogen. It cannot be isolated by ordinary laboratory techniques, only bacteria and other secondary organisms being obtained when pieces of seedling tissue, infected with *A. cochlioides*, are surface sterilized and plated out on agar media. If, however, portions of the infected seedlings are thoroughly washed and placed in Petri dishes containing 15 to 20 cc. of sterile water, after a few hours at room temperature *A. cochlioides* can be

detected by the thousands of evacuation tubes that it sends out and by the groups of encysted zoospores (Fig. 6). Zoosporangial formation and zoospore discharge can be readily observed in this water medium.

If a pure culture is desired, a Van Tieghem cell is placed in the center of a deep Petri dish immediately after a thick layer of warm 3% agar medium has been poured into it. The Van Tieghem cell has three little wires or pins clamped around it to raise it from the bottom of the Petri plate. Then a piece of tissue with some mycelial growth is rinsed in sterile water and pushed well down into the agar within the cell. The hard medium inhibits most bacteria from growing through it, and they develop only on the surface of the agar inside the Van Tieghem cell. The water mold, however, grows down through the medium, and emerges on the outside of the Van Tieghem cell free of bacteria and thus can readily be isolated in a pure state.

Life History of Aphanomyces cochlioides

Asexual reproduction can be induced without difficulty by immersing, in water, seedlings that are infected with *A. cochlioides* or young mycelia on a thin layer of cornmeal agar. After three or four hours hyphae can be seen radiating out from the infected tissue, and a little later, among them, tiny white specks, which are groups of encysted zoospores, can be observed with the naked eye. Under the microscope, short, predominantly unbranched, more or less sinuous threads will be found to project from the host. These efferent threads, which are evacuation tubes, are nonseptate, full of dense protoplasm, and from 8 to 10 μ in diameter and up to 2 mm. in length.

The protoplasm in the evacuation tubes and in the threadlike sporangia that are within the host, now breaks up into little rods about 12 μ long (Fig. 7). The first evidence of spore formation is the appearance of narrow transverse transparent bands across the protoplasm at regular intervals in these tubes. About five minutes later the sporangiospores flow rapidly from these tubes and sporangia and instantaneously round up, each spore surrounding itself with a thin cellulose membrane. Some sporangiospores vary from 6 to 15 μ, but are usually 8 to 9 μ in diameter and as many as 300 may aggregate at the end of a tube where, as Figs. 6 and 8 show, they look like bunches of grapes. A single group is shown in Fig. 9.

After three or four hours a small elevation is formed on each spore, and this gradually increases until within three to five minutes a papilla about 2 μ in diameter is produced. Then the membrane of this papilla breaks at its apex, and the protoplasm flows out rapidly through the necklike opening and forms

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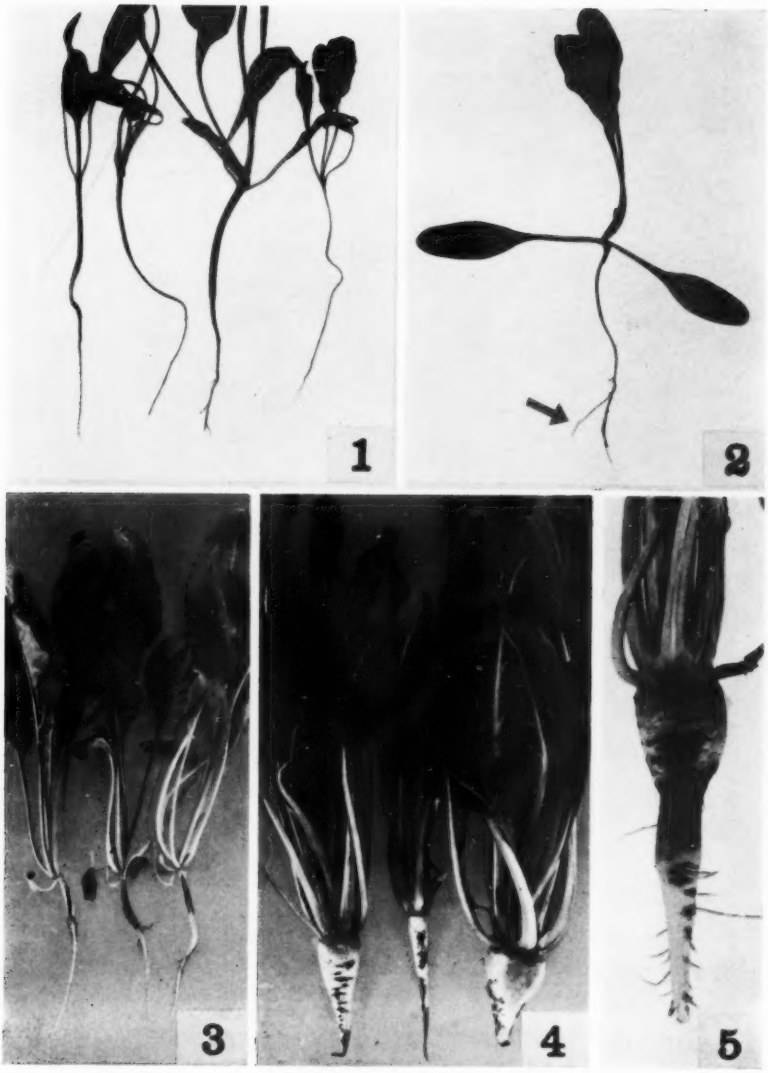
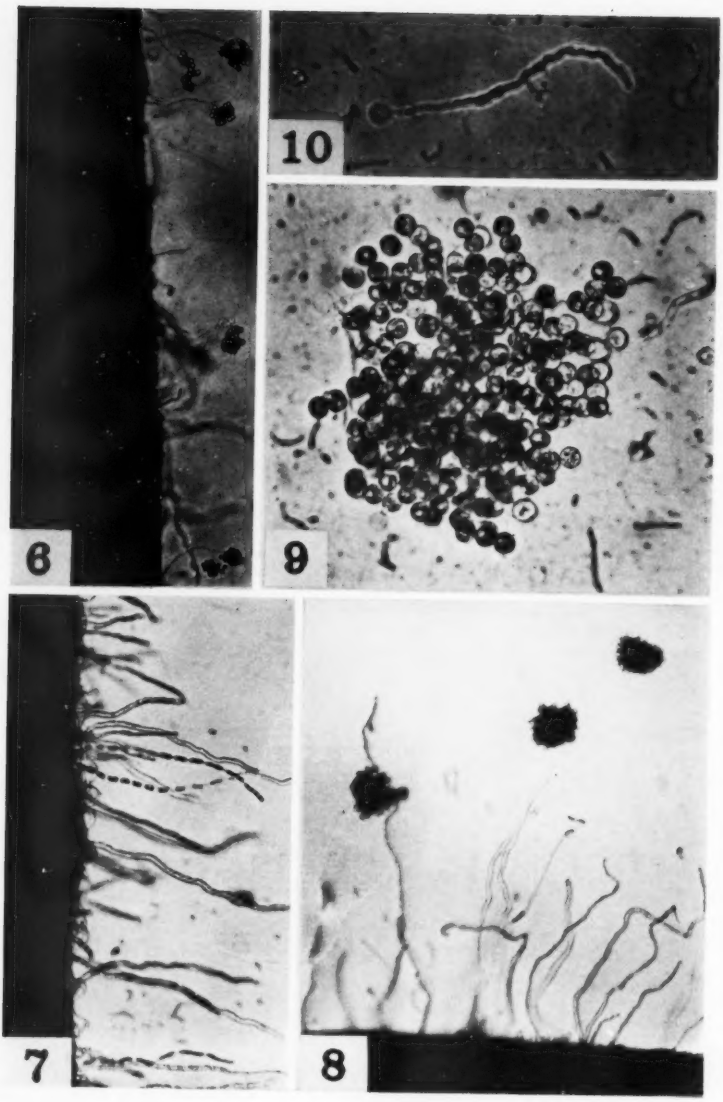
FIG. 1. *Seedlings showing acute symptoms of blackroot caused by A. cochlioides. From left to right: healthy; base of hypocotyl blackened; hypocotyl completely black; cortex completely destroyed and only vascular strand remaining.* FIG. 2. *A seedling recovering from blackroot; observe the new lateral root.* FIG. 3. *The cortex of the hypocotyl of the healthy beets has fissured and is beginning to slough off. The seedlings have three or four pairs of true leaves.* FIG. 4. *Beets suffering from the chronic phase of attack of A. cochlioides. Note that the root tips are necrotic.* FIG. 5. *A girdled beet, another manifestation of the chronic phase of blackroot.*

PLATE I

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another sphere, the cylindrical wall of the papilla persisting on the empty cyst membrane. Almost immediately two flagella, about 24 μ long, inserted in a slight depression of the spore, are detected waving in the water. The zoospores gradually increase their oscillating motion and the lashing of their flagella becomes more vigorous until after about 10 to 20 min. they are extremely active and tear away from the papilla membranes. The zoospores are usually 13 μ long and 7 to 8 μ in diameter, but large double spores have been observed. A whole group of encysted zoospores may be emptied in four or five hours. The zoospores swim vigorously for three or four hours, and then settle down and produce a germ tube, as is shown in Fig. 10. In distilled water the tubes may measure 2 mm. in length and occasionally they produce several branches some of which may reach a length of 70 μ.

The writer estimated that on a piece of infected hypocotyl 1 in. long and 1/16th in. in diameter as many as 500 evacuation tubes might be present, each of which would discharge on the average 100 zoospores. Thus from 0.003 cu. in. of infected tissue, 50,000 zoospores might be produced and liberated within one day. Each of these spores may attack a healthy seedling, and produce zoospores again within a couple of days, so that under favorable conditions the asexual stage may rapidly produce very abundant inoculum.

After zoospore production ceases, the sexual organs make their appearance, and finally are found scattered rather liberally throughout large portions of the collapsed host as is seen in Fig. 11. The oogonia (Fig. 12) are subspherical, smooth, usually 20 to 29 μ in diameter and provided with a wall of somewhat irregular inner contour about 1.6 μ in thickness. From one to four antheridia (Fig. 13), 6 to 10 μ in diameter and 10 to 18 μ in length, become wrapped about individual oogonia. The antheridia (Fig. 14) are club-shaped, separated from the stalk by a septum, and frequently are partly countersunk into an abrupt depression in the oogonial wall. In this region a short fertilization tube develops. The diameter of the oospore is about 5 μ less than that of the oogonium, and each contains an eccentrically placed reserve globule about 12 μ in diameter. The oospores are resistant to unfavorable climatic conditions and remain viable for a long time. They are shown to survive for one but not two years in air-dried, naturally-infested soil.

To find out whether *A. cochlioides* was homo- or heterothallic, a pure culture growing on cornmeal agar was placed in sterile water in a Petri dish. Zoospores that had encysted after they emerged from the sporangia were scattered over an agar plate from which individual ones were picked up by means of a biscuit cutter and transferred to another plate flooded with water.

FIG. 6. *A. cochlioides growing out from infected tissue, with groups of encysted zoospores at the tips of efferent tubes.* FIG. 7. *Numerous efferent tubes of A. cochlioides radiating out from the infected tissue. They are filled with dense protoplasm, which, in some, has broken up into rods or zoospores.* FIG. 8. *Groups of zoospores encysted at the tips of three efferent tubes and in one tube four zoospores have not escaped.* FIG. 9. *A single group of encysted zoospores at the tip of an evacuation tube.* FIG. 10. *A zoospore germinating after secondary encystment.*

In every case the colonies resulting from individual zoospores produced normal sexual fruiting bodies. *A. cochlioides* is accordingly homothallic, unless perchance within the species, strains of different potentialities exist.

Host–Parasite Relations

From freehand sections of living material the development of the fungus has been traced in the seedling. Mycelium has been found only in the intercellular spaces of the cortical tissue of the hypocotyl and root before the cortex is sloughed off (Fig. 15). The hyphae are rather scanty in affected tissues and a relatively small portion of the intercellular spaces is occupied. *A. cochlioides* spreads slightly in advance of visible symptoms and may migrate up to the hypocotyl into the cotyledonary leaves. The cell walls adjacent to the mycelium assume a dark brown to black color and, when stained with cotton blue, have a greenish yellow appearance. Sometimes in the later stages of infections considerable amount of blackish granular material may surround the hyphae in the intercellular spaces.

Observational evidence indicated that the pathogen probably entered through the hypocotyl since necrosis consistently appeared first on its lower part. To test this, seedlings were transplanted to 4-in test tubes into which two small holes had been blown, one in the bottom and the other one about one inch and a half up the side. Through these air and water could enter freely. Sterile soil was then placed around the root and a 50% mixture of vaseline and low-melting paraffin was poured around the seedling so that the top of the root and the bottom of the hypocotyl was sealed off (Fig. 16). Inoculum, in the form of pieces of infected seedlings or bits of agar containing *A. cochlioides*, was placed sometimes below and sometimes above the paraffin–vaseline seal; other seedlings were left uninoculated as checks. About one-half inch of water was then poured around the hypocotyl and the test tubes were placed in a beaker that contained about an inch of water.

In all test tubes in which the inoculum was introduced above the paraffin–vaseline seal, typical blackroot symptoms of the beets occurred and the plants soon died as a result of infection by *A. cochlioides*. When inoculum was introduced below the seal, blackroot symptoms did not appear within three weeks, at which time the experiment was discontinued. Of the 87 plants that had inoculum introduced with the roots, 16 died soon after transplanting, but from none of them could *A. cochlioides* be isolated. However, approximately the same percentage of check plants also died immediately after transplanting, owing, apparently, to some mechanical injury or the use of a paraffin–vaseline mixture that was too warm. Thus it appears that penetration is limited to and takes place readily through the hypocotyl. There are numerous open stomata on the hypocotyl of the sugar beet and these may well be the portal of entry for *A. cochlioides* since the hypocotyl has a thick cuticle and since the pathogen always appears to be intercellular.

In greenhouse experiments the chronic phase has never been observed on beets growing either in naturally- or artificially-infested soil, even when the soil

PLATE III

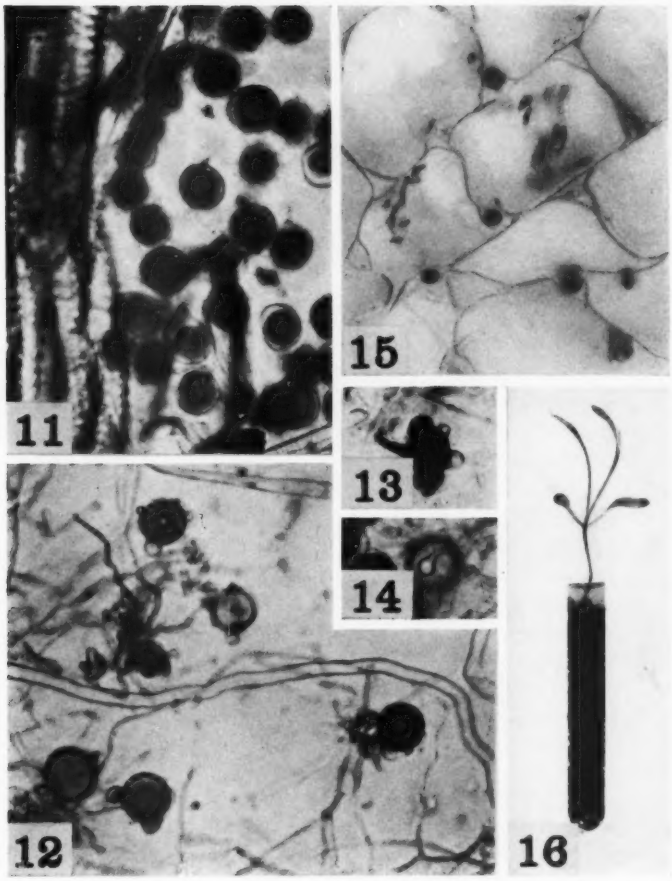


FIG. 11. *A group of oogonia of A. cochlioides in the cortex of the infected beet.* FIG. 12. *Oogonia at various stages of development; lower left, oogonium prior to oospore formation; immediately to right, oogonium with oospore; at right an oogonium within which there is an oospore with an eccentrically placed reserve globule.* FIG. 13. *Oogonium with four antheridia attached.* FIG. 14. *A club-shaped antheridium separated from the stalk by a septum.* FIG. 15. *A section of cortical tissue showing the mycelium in the intercellular spaces.* FIG. 16. *A sugar beet seedling showing blackroot symptoms. Inoculum was introduced above the seal of paraffin and vaseline.*

is kept saturated. In January 1948, inoculum was placed in artificial wounds made in beets about three months old. A plug about $\frac{1}{4} \times \frac{1}{4} \times \frac{1}{4}$ in. was removed, inoculum inserted, and the plug replaced. In all 16 beets so treated no rotting of the roots occurred and the plants remained free from disease as did also the checks. The inoculum that was used in this experiment was originally obtained from a seedling that had suffered from the acute phase of attack and thus this strain was capable only of producing seedling infection.

Humidity Studies

Since infected seedlings have frequently been observed to recover and the fungus is intercellular in the host tissue, it seemed likely that humidity might be a limiting factor in the parasitic activity of *A. cochlioides*. An attempt was made to evaluate this possibility.

An apparatus was set up to test the lowest humidity at which this fungus would grow. Saturated air was passed through appropriate solutions in a series of Pettenkoffer tubes, from each of which the air emerged at a definite humidity (9). The air was blown over the fungus, which was growing on a thin piece of agar on the side of a test tube. By this method it was found that the growth of the fungus ceased when the relative humidity dropped to 95% or below. For more precise measurements another apparatus was used.

Further studies were carried on in Petri dishes in an incubator at 25° C. About 50 cc. of sulphuric acid of desired concentration (16) was poured in the tops of Petri dishes. Other inverted Petri tops, containing a thin layer of potato dextrose agar, were placed exactly over the acid dishes and the two tops were then sealed with vaseline. After two weeks, the plates were inoculated with *A. cochlioides* on a small piece of agar. Growth continued for a very limited time at all humidities, but soon stopped at a humidity of 98.7% whereas at 99.1% the fungus grew across the plate in all cases.

To check the above results, infected seedlings were placed in 0.0, 0.1, 0.2, ... 1.0 *M* solutions of sucrose at 25° C. Development of the fungus gradually diminished from the 0.2 to 0.6 *M* solution in which there was no growth. When the infected seedlings were taken from the 0.6 molar solution and put in sterile water, no growth occurred, the fungus apparently having been killed. A 0.6 *M* sucrose solution has an osmotic pressure of 15.6 atmospheres and an equivalent relative humidity of 99.0% (15). These results therefore correspond fairly closely with those obtained with the acid solutions. Since the humidity of the intercellular spaces of plants may be below 99% (17), humidity may frequently limit the development of *A. cochlioides* when the soil and atmosphere become dry.

Temperature–Growth Relations

Since field evidence indicated that temperature was frequently a limiting factor in the development of blackroot, the temperature relations of *A. cochlioides* and of disease incidence were investigated. The fungus was grown at temperatures of 5°, 10°, 13°, 16°, 20°, 22.5°, 25°, 27°, 29°, 31°, 33°, 35°, 37°,

40°, 43° C. on a thick layer of potato dextrose agar in order that the air and media would remain moist throughout the experiment. Fig. 17 shows the average diameter of the colonies after 48 hr. A parallel series on cornmeal

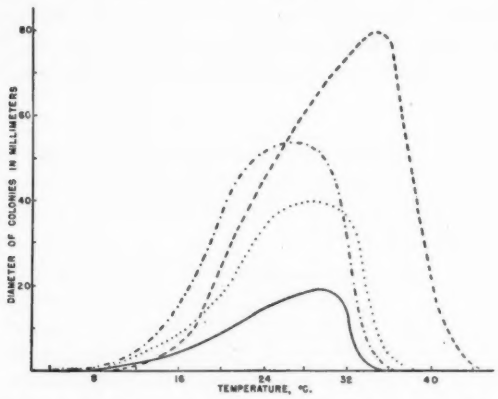


FIG. 17. *Influence of temperature on the rate of growth of the four pathogens on potato dextrose agar; A. cochlioides* ——————; *R. solani* . . . . . ; *P. ultimum* -·-·-·-·-; *P. aphanidermatum* – – – – –.

extract agar gave similar results. However, it is believed that the vegetative development of *A. cochlioides* may be only a relatively unimportant phase in its life history and that the mycelium very infrequently grows through the soil since vegetative hyphae are never sent out from diseased seedlings when they are placed in water and since this organism is only pathogenic in wet or saturated soil. It was apparent that the abundance and rate of production of zoospores should also be compared at the various temperatures. Infected seedlings were placed in sterilized water in Petri plates at the above temperatures and the times required for primary zoospore encystment, for the release of the zoospores from the cyst, and the germination of the zoospores were recorded. All three of these measurements followed the same general curve. The rate of primary zoospore encystment that could be measured most accurately is shown in Fig. 18.

The optimum temperature for mycelial growth was at least 6° C. higher than the optimum temperature for spore formation. It was observed also that the number of sporangia and zoospores formed diminished markedly on either side of this optimum temperature. This curve furthermore cut away very sharply at its limits. Since both the number of spores and the rate of their production are very important in disease spread, and since mycelial growth must occur within the host, both should be correlated with pathogenicity.

In Wisconsin temperature tank experiments naturally-infested blackroot Brookston clay loam soil was used and temperatures were maintained at

5°, 9°, 13°, 17°, 21°, and 25° C. In each tank two canisters of soil were kept dry, three at optimum moisture content, and three were kept moist, the moisture content being 20, 30, and 43% dry weight, respectively. Sixty

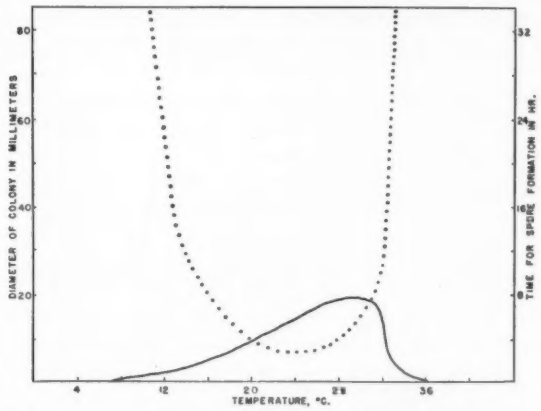


FIG. 18. *Influence of temperature on the time required for A. cochlioides to produce groups of encysted zoospores following the immersion of infected seedlings in water . . . . . ; the influence of temperature on mycelial development* ————————.

sugar beet seeds were planted in each canister and the experiment was repeated. Seeds were soaked in water a day previous to planting in order that emergence might be uniform in the dry soil.

As Table II shows, no blackroot occurred in the dry soil but as the moisture content was raised, the disease became more severe. At 13° C. and below there was only a slight amount of injury but at 17° C. blackroot was becoming

TABLE II

THE PERCENTAGE BLACKROOT IN NATURALLY-INFESTED BROOKSTON CLAY LOAM SOIL AT VARIOUS TEMPERATURES AND MOISTURES

Soil moisture	Temperature, ° C.					
	5	9	13	17	21	25
Dry – 20% water content	0	0	0	0	0	0
Optimum – 30% water content	0	1	0	6.4	8.1	11.5
Moist – 43% water content	0	1.3	4.4	18.8	32.5	30.1

serious and at 21° and 25° C. it was very severe. Judging from these results the rate of spore production appears to be most directly related to disease incidence.

Epiphytotics

In Ontario, blackroot usually occurs in late spring epiphytotics. At this time serious outbreaks can be expected a few days after a rain if in the meantime the weather remains warm. From this it was apparent that a high soil temperature and abundant moisture are necessary for disease development. Fig. 19 gives the daily rainfall, soil temperature, and disease incidence during

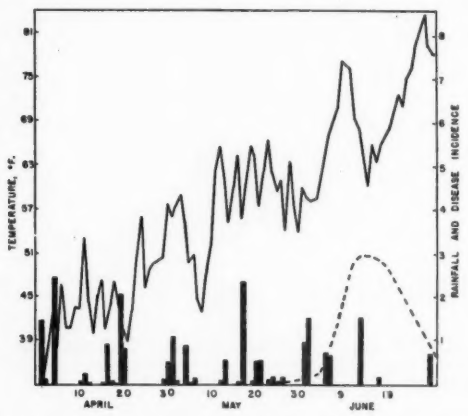


FIG. 19. *The influence of soil temperature and rainfall on disease incidence, one inch of rainfall being equal to 2;* – – – – – – *disease incidence, approximate acreage initially attacked at the times indicated, the ordinate 1 = 600 ac.;* ———————— *temperature.*

April, May, and June in the spring of 1947. Rainfall was frequent and abundant except for a short time at the end of June, when moisture became a limiting factor. Beet seed was planted more or less throughout the whole period and thus there were seedlings in the susceptible stage at all times. Inoculum was present in a great many of the fields, so that temperature apparently was the limiting factor until about June 8, when a serious disease outbreak began. Previous to this date the soil temperature had exceeded 60° F. only a few times and for very short periods. Below 60° F. *Aphanomyces*, as Fig. 18 shows, develops very slowly, whereas at higher temperatures the growth rate increases very rapidly. From the 7th to the 14th of June, conditions were ideal for a disease outbreak inasmuch as the soil temperature was high, moisture abundant, and many of the seedlings were in their susceptible period. Later in June the outbreak decreased because the soil was drier and the seedlings were passing into a resistant stage. In all the blackroot epiphytotics that have been under observation the same interplay of soil moisture, temperature, and host disposition has been apparent.

Host Range of A. cochlioides

Some of the wild and cultivated plants that occur commonly in the sugar beet district were tested for susceptibility in sterile soil inoculated with

A. cochlioides. Sugar beet seed was planted along with the other seeds to ensure that *A. cochlioides* was present. Only lamb's quarters (*Chenopodium album* F.) and pigweed (*Amaranthus retroflexus* L.) were susceptible. *Chenopodium album* is in the same family as *Beta vulgaris* and becomes resistant at about the same stage in its development, but *Amaranthus retroflexus* is in the *Amaranthaceae* and is susceptible only during the first two weeks of development. Alsike, alfalfa, white Dutch clover, yellow and white sweet clover, red clover, sweet pepper, soybean, garden pea, wild carrot, dandelion, barley, fall and spring wheat, oats, yellow foxtail, timothy, and sweet corn were found to be immune. Since blackroot is only destructive in fie ds that have continually or frequently been planted with sugar beets, it appears that the weed hosts are not very effective in the build-up of this organism.

Seedling Blight Caused by *Pythium aphanidermatum*

Symptoms

Pythium aphanidermatum attacks the seedling during its primary development, and may cause pre-emergence or postemergence blight. The embryo may be invaded before it has had time to germinate, and frequently the seedling is attacked before the cotyledons push out of the soil. The fungus may even attack the cotyledonary leaves, but more frequently it is the young root that suffers from primary infection. In a few hours after invasion the whole seedling has a water-soaked appearance and is killed.

Postemergence blight also proceeds very rapidly and 24 hr. after the first indication of wilting the seedling is almost completely decayed. Most of the seedlings are infected a day or two after emergence. Infection begins in the root, and as the moist decay advances up the hypocotyl, the seedling wilts, in a short time falls over, dries, and shrivels so completely that it is soon difficult to find any remnant of it.

If the seedlings become infected a few days later or when they have begun to produce their first pair of true leaves, they display different symptoms. In most seedlings the disease progresses so rapidly that the plant collapses at the ground level and falls over before the seedling has begun to wilt. In some cases, however, the seedling may be held upright by the surrounding soil, the cotyledonary leaves meanwhile becoming bluish in color, and a day or two later the desiccated plant falls over. In all these plants the roots are completely destroyed. The disease symptoms are shown in Fig. 20.

Life History of Pythium aphanidermatum

It was found that *P. aphanidermatum* could be readily isolated from infected tissues by the method already described for the isolation of *A. cochlioides*. It can be isolated also on agar media, but this involves more materials and labor than the water culture method, and several days are required to identify the pathogen, whereas in water it can be recognized within a day.

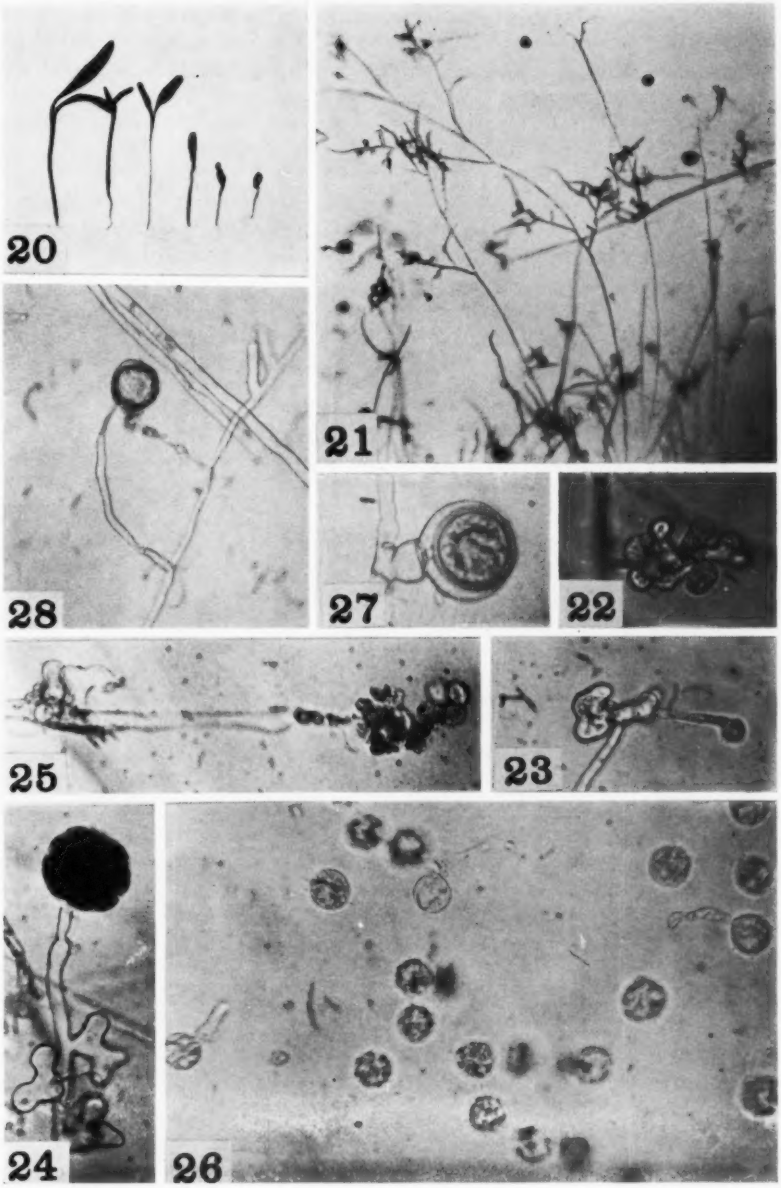
If a seedling, infected with *P. aphanidermatum*, is placed in water, on its surface abundant branched nonseptate mycelium begins to form at once, and within a few hours irregular lobulate inflated sporangia of varied size are present (Figs. 21 and 22). These sporangia mature very rapidly; protoplasm can be observed flowing in the mycelial threads and is shunted into the many enlarging sporangia. On each sporangium an evacuation tube forms (Fig. 23), and at its tip a vesicle arises (Fig. 24). The protoplasm from the sporangium flows into the vesicle and within a minute its development may be completed. The protoplasm in the vesicle is at first very dense and uniform in appearance and is in continuous motion. After a couple of minutes the zoospores are delimited and about four minutes later movement of the individual zoospores begins and soon a vigorous lashing of flagella can readily be detected. The activity of the spores increases extremely rapidly, they jostle violently within the vesicle, and in a minute or two the vesicular membrane breaks; the zoospores escape in rapid succession (Fig. 25) and swim swiftly in all directions. They are about 12 μ long and 7.5 μ wide and have two lateral flagella. After swimming for a few hours, the zoospores encyst and then germinate in the manner shown in Fig. 26.

It was observed that immediate germination of the sporangia took place only when the infected seedling was placed in a small amount of water in the Petri dish so that the mycelium and asexual reproductive organs were floating at or near the surface of the water. If the diseased tissue is immersed in water so deeply that the mycelium and the sporangia are completely submerged, germination is greatly retarded and frequently suppressed.

The above description of asexual reproduction is not applicable to spore discharge at low temperatures. At 10° C. or slightly below, the zoospores after delimitation do not begin to move but remain quiescent within the vesicle for several minutes. The vesicle then breaks, the motionless zoospores merely drift away, and do not germinate as long as this low temperature is maintained. At 12° C. the normal method of zoospore discharge is carried on, and the spores are motile. Thus it appears that the breaking of the vesicle is not the result of the bombardment with zoospores, because here the vesicle bursts even though the zoospores remain motionless.

After one or two days asexual reproduction stops and sexual organs begin to appear abundantly on the external mycelium, but they usually do not develop within the host as was so common in the case of *Aphanomyces*. A

FIG. 20. *Seedlings killed by P. aphanidermatum blight. The seedling on the right suffered from pre-emergence blight and even the cotyledonary leaves were infected. The oldest seedling on the left suffered only from root infection.* FIG. 21. *Mycelium, sporangia, and vesicles of P. aphanidermatum produced soon after an infected seedling is immersed in water.* FIG. 22. *A typical sporangium.* FIG. 23. *A sporangium with an evacuation tube and at its tip a vesicle beginning to form.* FIG. 24. *A newly-formed vesicle in which the zoospores are just beginning to be delineated. The empty sporangium and evacuation tube is also shown.* FIG. 25. *Zoospores breaking out of a vesicle.* FIG. 26. *A group of encysted zoospores and some that have germinated.* FIG. 27. *An oogonium and an intercalary antheridium with its penetration tube.* FIG. 28. *An apical oogonium and antheridium.*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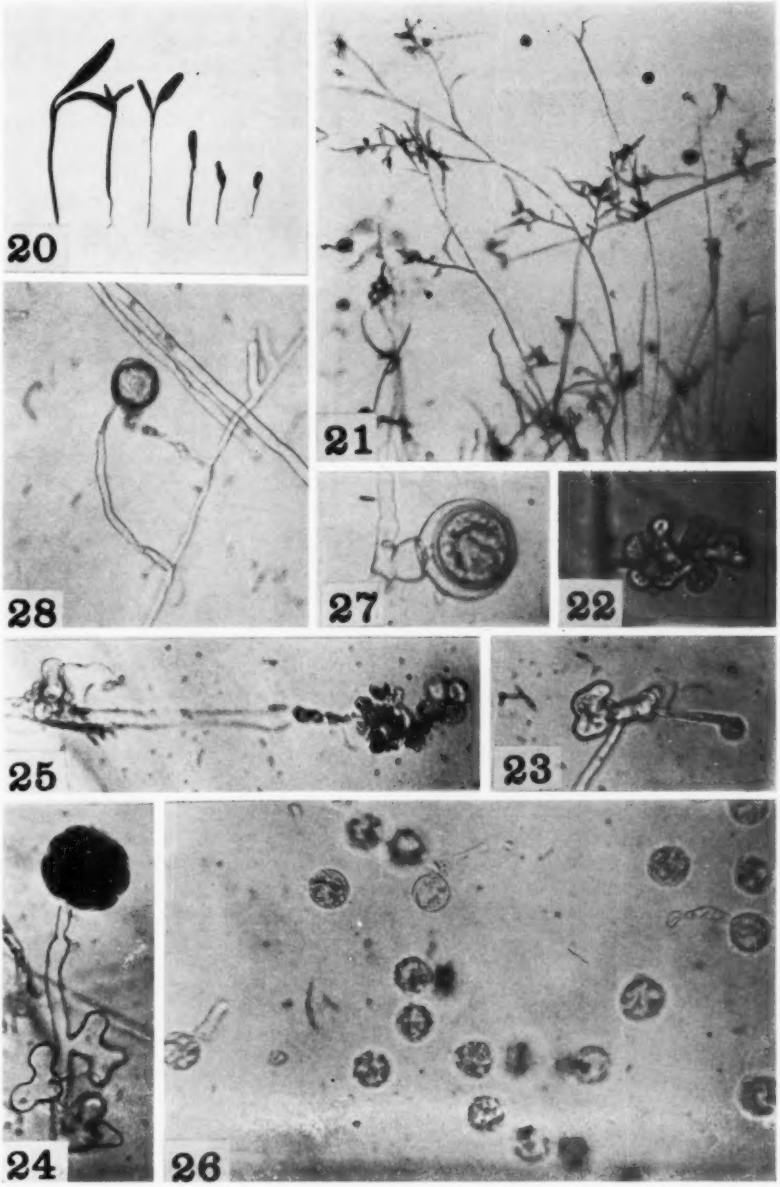
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typical intercalary barrel-shaped antheridium with a conspicuous penetration tube is shown in Fig. 27. They measure 9-11 × 10-14 μ, are usually monoclinous but sometimes are diclinous and one or two are present with each oogonium. The oogonia, which are generally terminal (Fig. 28), but occasionally intercalary, are 22 to 27 μ in diameter. The oospores are aplerotic, 17 to 19 μ in diameter, and germinate by a germ tube.

Host–Parasite Relations

Colonization of a seedling by *Pythium* is achieved through the following sequence of events. A zoospore comes to rest on the surface of a root, produces a germ tube, and soon an appressorium forms on the epidermis. A penetration tube develops at the base of the appressorium and pushes through the epidermal wall where it enlarges and continues to grow intracellularly through the cortex and even into the stele. The mycelium is constricted where it passes through the cell walls and is to be found in advance of the necrotic symptoms.

Seedlings grown at a low temperature are susceptible for a considerably longer time than those produced at high temperatures but in any case they become resistant when the first pair of true leaves are about ¼ in. long, at which time, according to Artschwager (1) primary growth of the beet is concluded. This resistance might be of a physiological or a mechanical nature. If it were mechanical it would appear to be associated with the development of a suberin lamella over the entire surface of each endodermal cell, with the exception of the root tip, which retains a primary endodermis. To enquire into this possibility, seedlings were grown in sterile soil that was saturated with water so that oxygen would be excluded from the roots. It was hoped that, in the absence of oxygen, fatty acids could not be changed into suberin, in which case the roots might still be susceptible. After the seedlings had passed their usual susceptible stage, *P. aphanidermatum* inoculum was put in the soil but no blight occurred. However, it was found that suberin did form in the roots that were growing in water. Even if the fungus could not penetrate the suberin lamella, entrance should still be possible through the root tips that are unsuberized, through transfusion cells in the older epidermis, or through wounds produced by the emergence of secondary roots. This suggests that resistance must be due to some physiological condition that arises at the beginning of secondary growth.

Epidemiology

Both high soil temperature and abundant moisture are necessary for an epiphytotic of blight in the Exeter sandy loam soil. In spite of this, and although the seedlings are susceptible to *P. aphanidermatum* for only a very short time, nevertheless whole fields of sugar beet seedlings may be destroyed.

The necessity of abundant moisture is apparent since only under such conditions could zoospore production or liberation occur. The growth–temperature curve for mycelial development (Fig. 29) shows further that

high temperature likewise favors rapid growth of *P. aphanidermatum*, whose optimum temperature of 34° to 35° C. is unusually high and at least 6° to 8° C. higher than the optimum of the other sugar beet pathogens studied. How-

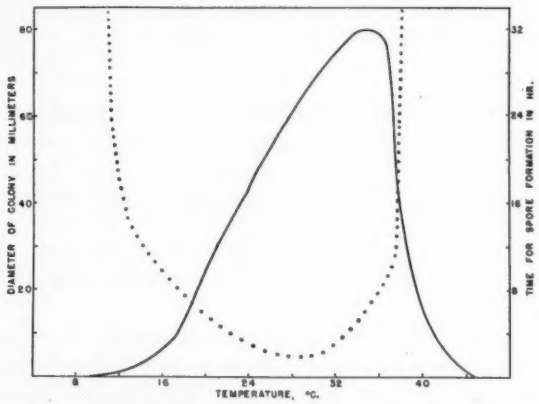

FIG. 29. *Influence of temperature on the length of interval required for P. aphanidermatum to produce zoospores from the time infected seedlings are immersed in water . . . . . ; the influence of temperature on mycelial development ————————.*

ever, *P. aphanidermatum* develops very rapidly over a wide range of temperatures with its lower limit only slightly higher than that of the other pathogens (Fig. 17).

Since the zoospores play such an important part in the spread and build-up of this pathogen, the time required for zoospores to form at various temperatures after naturally-infected seedlings were placed in water was determined. The curve representing the rate of zoospore production is shown in Fig. 29 and its optimum is much wider and about 6° C. lower than that for mycelial development. At temperatures on either side of the optimum, the time required for zoospore production increased and also the number of sporangia and zoospores formed diminished extremely rapidly. For example, at 16° C. there was only about one-fifth as many sporangia formed as at 29° C.

Pathogenicity of this organism was tested in naturally-infested sandy loam soil in Wisconsin temperature tanks. The soil was kept very moist throughout the experiment and air temperature was maintained at about 27° C. Sixty seeds were planted in each of eight canisters of all tanks.

The average results of two experiments are given in Table III from which it is apparent that the greatest amount of blight occurred at 25° and 30° C. with only a slight amount at 15° and 37° C. At 10° C. no blight occurred, which was to be expected since at this temperature the fungus grows very slowly.

TABLE III

EFFECT OF TEMPERATURE ON THE INCIDENCE OF SEEDLING BLIGHT IN MOIST SANDY LOAM SOIL NATURALLY INFESTED WITH *P. aphanidermatum*

Temperature, ° C.	Postemergence blight, %	Final stand, %
37	6.9	90.8*
30	36.2	48.7
25	33.9	45.1
20	27.0	61.3
15	4.1	94.1
10	0.0	98.2

* *Germinating capacity of seed 98%.*

In these experiments blight reached a maximum within a day or two after the day on which the largest number of seedlings emerged. This correlation between emergence and blight has been consistently encountered in flat experiments.

Diseases Due to Other Pathogens

Pythium ultimum

Disease symptoms resulting from *P. ultimum* attack resemble those on seedlings infected with *P. aphanidermatum* and again the seedlings become resistant when secondary growth begins. *P. ultimum* is a low temperature fungus, as Fig. 17 shows, and it may cause blight even at 5° C.

The pathogen is readily identified in water and it produces many sporangia and oogonia on the mycelium that passes out from the host (Fig. 30). The sporangia are usually terminal, about 20 μ in diameter and always germinate by a germ tube. The oogonia (Fig. 31) are spherical, generally terminal, and approximately 21 μ in diameter and contain a single aplerotic oospore that has a single central reserve globule and a refringent body. There is usually one antheridium per oogonium; it is curved, monoclinous, and arises immediately beneath the oogonium.

P. ultimum was found in all the clay soils studied and even in the marl soil of the Blackwell area but in spite of this wide distribution it is of little economic importance. This organism may cause a considerable amount of blight in the greenhouse but during the past three seasons it has not caused serious damage in any sugar beet field. In the marl soil, rootrot is not a problem whatsoever but under greenhouse conditions *P. ultimum* can be readily isolated from infected seedlings and this may be due to the higher soil temperature under which the beets are grown.

Rhizoctonia solani

This organism is also widely distributed and of little economic significance. However, it may infect beets at any age, the point of attack usually being at the ground level (Fig. 32). On seedlings it produces dry local lesions that

completely cut off the beet at the soil line. On lesions that may occur on large beets the mycelium can frequently be seen. This fungus grows well on infected tissue in water and can be identified by the angle the branches form to the main hypha, the constriction of the branches where they join the main thread, and the uniform placement of the first septum in the branch (Fig. 33).

Although *R. solani* isolates were extremely virulent in flat tests in which the soil had been sterilized previous to planting and despite the wide distribution of this pathogen, it caused an exceedingly small amount of rootrot. This suggests that some other soil organisms may antagonize or prohibit the development of *R. solani* especially since Weindling (19) found that *Trichoderma lignorum* antagonized the development of *R. solani* and Warren (18) frequently isolated from fragments of diseased beets a species of *Papulospora* along with *R. solani;* this species, he showed, was parasitic on *R. solani* and effective in reducing the amount of sugar beet rootrot *R. solani* caused. Low temperature may also limit the parasitic activity of *R. solani* in the field because the soil temperature during seedling development is usually below 65° F. and this fungus develops only at one-third of its optimum rate at this temperature. This is supported by the fact that Leach (11) states that in Central California this fungus is rarely recovered from early spring plantings but frequently from later ones.

The Distribution of Pathogen in Relation to Depth and Soil Type

To investigate the abundance of pathogens at different depths, soil samples were taken from four profiles in two Brookston clay loam rootrot fields, and from four profiles from Exeter sandy loam. For each profile, holes were dug about 2½ ft. deep and one side was smoothly squared perpendicular to the soil's surface. Soil samples were removed by means of a cork borer at 2-in. intervals and in each profile the dividing line between the top and subsoil was noted. Inasmuch as these pathogens cannot be isolated directly from the soil, the number of seedlings that damped-off in these soil samples was used as the measure of the abundance of the pathogens. The soil samples were put in 2-in. glass caps; sugar beet seed was planted in each and in order that rootrot would be at its maximum the soil was kept very damp.

In the Exeter sandy loam the top soil was approximately 8½ in. thick and *P. aphanidermatum* produced blight equally readily in all samples taken from this layer, whereas none occurred in the subsoil. Dilution plates showed that

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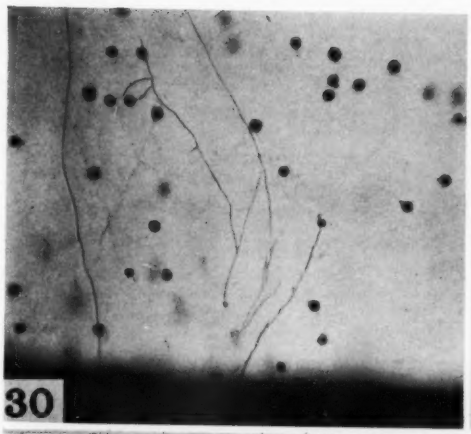
FIG. 30. *Asexual and sexual organs on the mycelium of P. ultimum soon after infected seedlings were placed in water.* FIG. 31. *A typical antheridium and oogonium of P. ultimum.* FIG. 32. *Seedlings that have suffered from R. solani attack. Infection occurred at the ground level.* FIG. 33. *Typical R. solani mycelium, note the constriction at the origin of the branch, the 45° angle that it forms with the main thread and placement of first septum, in branch.* FIG. 34. *Response of sugar beets in increased stand and enhanced vigor to the incorporation of soybean residue (Flats 1) as contrasted with untreated soil (Flats 2).* FIG. 35. *A. cochlioides growing around a lump of Arasan on a Petri plate.* FIG. 36. *The retardation in growth of P. aphanidermatum is shown as it approaches the Arasan.*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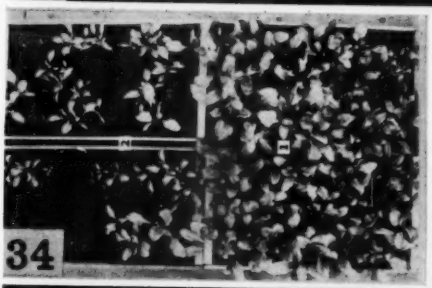
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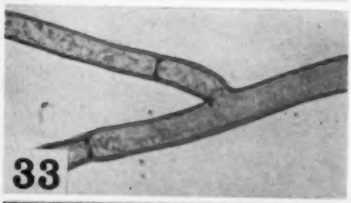
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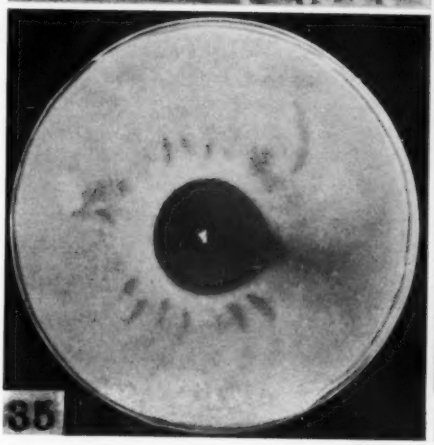
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bacteria were twice as numerous and fungi 50 times as abundant in the surface soil as in soil 13 in. deep. The dilution plate technique in described in the succeeding section.

In the Brookston clay loam soil *A. cochlioides* and *P. ultimum* likewise were found only in the top soil and, as shown in Table IV, were most abundant in the top two inches of surface soil.

TABLE IV

FREQUENCY OF ISOLATION OF *P. ultimum* AND *A. cochlioides* AT DIFFERENT DEPTHS IN ROOTROT BROOKSTON CLAY LOAM SOIL

Depth, in.	*P. ultimum*	*A. cochlioides*
½	112	72
2	97	54
4	71	25
6	26	20
8	20	0

As was indicated in Table I the distribution of sugar beet pathogens was closely correlated with soil type; thus *A. cochlioides* was isolated from clay soil only, *P. ultimum* from the clay and marl soils, and *P. aphanidermatum* from the Exeter sandy loam in which it was the only pathogen present.

Although *P. ultimum* and *A. cochlioides* are not found in the sandy loam soil, under greenhouse conditions they will survive in it along with *P. aphanidermatum* for at least one year. It is difficult to understand why they are not present in this sandy loam. However, since sugar beets have only been grown in this area for a few years perhaps sufficient time has not elapsed for these two pathogens to increase in abundance or to have spread into this area. This explanation does not seem too satisfactory inasmuch as *P. aphanidermatum* is already very abundant. However, the build-up of *P. aphanidermatum* may have occurred on some other host that is commonly grown in this locality, or possibly *P. aphanidermatum*, which is one of the high temperature *Pythium* spp. (13), is better adapted than the other pathogens to the high temperatures that prevail in this light sandy soil.

To test the survival of *P. aphanidermatum* in clay soils a cornmeal sand culture was mixed with some University of Toronto clay in October 1947. Abundant blight of sugar beets, which were planted in it immediately after inoculation, occurred. The soil was then divided in five portions; two were put in a room at about 25° C. and one portion was kept moist while the other one was allowed to dry; two were treated likewise in a 5° C. room; and one portion was put outside. An equal quantity of Brookston clay loam soil received the same treatment.

In April 1948 the presence of *P. aphanidermatum* in these soils was tested by planting sugar beet seed in them. The soil was kept moist in order to increase the amount of blight, and the Brookston clay loam soil was put in

canisters in a Wisconsin temperature tank maintained at 35° C., so that no rootrot would be produced by the lower temperature pathogens. Since blight did not occur in either soil, *P. aphanidermatum* evidently did not survive six months in these clay soils. Since the persistence of specific fungi in a given soil environment seems to be the resultant of a large number of physical, chemical, and biotic factors, it would require substantial and far-reaching investigations to determine in this case what is basic to the correlations between soil types and pathogen distribution. It is felt, however, that the evidence presented strongly indicates that the correlations are authentic and the distributions hence are likely to remain functions of the soil types.

Soil Microbiological Studies

The soil in the vicinity of the roots usually supports noticeably higher numbers of microorganisms than soil more distant from the plant. The zone close to the roots and under the influence of their excretions has been called the rhizosphere. With strawberry rootrot it has been found that the flora in the rhizosphere of diseased roots is greater than that of healthy roots (8). It appeared that a study of the rhizosphere of beet roots in relation to infection might be of value.

Using the standard technique, rhizosphere studies were made of healthy roots and ones just beginning to show disease symptoms, on the 8th, 11th, and 14th day after planting. The seedlings were grown in Brookston clay loam and were watered moderately. Dilutions of 1 : 10,000 were plated for fungus counts in Petri dishes of peptone–glucose agar (5), and Czapek's agar modified by reducing the sucrose content to 1%. The peptone–glucose Czapek's agars were sterilized in Erlenmeyer flasks that contained 100 cc. of media and, preparatory to pouring in Petri dishes, the pH was adjusted to approximately 4 by the addition of $\frac{1}{2}$ cc. of normal sulphuric acid to each. Dilutions of 1 : 1,000,000 were plated in Petri dishes of sodium albuminate (5) for counts of bacteria and actinomycetes.

The results are summarized in Table V, which shows that bacteria were much more numerous in the rhizosphere of the diseased than the healthy roots and the difference became more marked as the plants became older. The number of fungi in the rhizosphere of diseased seedlings was significantly lower than in that of the healthy ones on the eighth day and no appreciable variation in number occurred later. Furthermore, in the rhizosphere of the healthy seedlings the number of fungi declined as the seedlings grew older unti' on the 14th day they were less abundant than in the case of the diseased seedlings. Inasmuch as the symptoms at that stage are indistinguishable, the diseased seedlings might have been infected with either *A. cochlioides* or *P. ultimum* or both and each of these might have exerted a specific but markedly different influence on the rhizosphere. It is believed, however, that *P. ultimum* caused most of the infection since the soil was watered moderately and the seedlings were young.

TABLE V

COMPARISON OF THE RHIZOSPHERE POPULATION OF DISEASED AND HEALTHY SUGAR BEET SEEDLINGS AT DIFFERENT AGES GROWN IN BROOKSTON CLAY LOAM

Age of seedling in days	Diseased seedlings			Healthy seedlings		
	Fungi on:		Bacteria and actinomycetes	Fungi on:		Bacteria and actinomycetes
	Peptone–glucose agar	Czapek's agar		Peptone–glucose agar	Czapek's agar	
8	22*	20	137**	37	33	99
11	27	19	159	19	14	87
14	17	21	283	13	14	115

** Number of fungi in $1/10^4$ gm. of soil; ** number of bacteria in $1/10^6$ gm. of soil; each is the average count in five Petri plates.*

The cause of the increased numbers of bacteria in the rhizosphere of seedlings immediately after the initiation of infection is by no means obvious. It could result from some common influence exerted by the pathogen; it might reflect merely local increases in nutrients associated with the necrosis of the roots, or it might be due to quite unrelated factors or to a combination of several of them. It seemed, however, that a study of the rhizosphere of roots that were killed artificially might aid in evaluating the influence of the necrotic factor in comparison with that of the parasite.

Sugar beet seed was planted close to the glass plates in miniature root-observation boxes (12), which were about 3 in. high, 1 in. thick, and 3 in. long. The glass plates that formed the two sides of these boxes fitted in wooden grooves on either side and could readily be pulled upwards, thus exposing the roots on the side, in the region of the seed. At about the soil line, the hypocotyl of the seedling was frozen for a few seconds over a distance of one-quarter of an inch. Liquid carbon dioxide was used for this purpose, because it left no chemical residue and had no affect on the soil flora. The freezing was done by touching the hypocotyl with the convex side of a pipette whose tip curved through 180° and through which carbon dioxide passed causing ice to form on its surface. Since this ice did the freezing the gas stream did not touch the seedling or the soil. This treatment was carried on between the 10th and 25th day after planting in Exeter sandy loam soil in which only the one pathogen *P. aphanidermatum* was present.

Rhizosphere studies were made one, two, three, and four days after treatment and in one series parallel rhizosphere counts were made of healthy seedlings (Table VI). In the first series both fungi and bacteria increased in the rhizosphere of the frozen seedling from the time of treatment. With the fungi this was not very marked, but the increase in the number of bacteria and actinomycetes each day was enormous and reached 700% within three days. In the second series, rhizosphere studies were again made of both healthy and frozen seedlings after periods of one, two, three, and four days. The first

TABLE VI

COMPARISON OF THE RHIZOSPHERE POPULATION OF SUGAR BEET SEEDLINGS GROWN IN EXETER SANDY LOAM, ONE, TWO, THREE, AND FOUR DAYS AFTER LOCAL FREEZING

Days after treatment	Fungi on:				Bacteria and actinomycetes	
	Peptone–glucose agar		Czapek's agar		Frozen	Healthy
	Frozen	Healthy	Frozen	Healthy		
1	18*	—	13	—	37**	—
2	23	—	16	—	178	—
3	24	—	24	—	246	—
1	74	86	70	68	93	91
2	36	52	37	48	135	65
3	68	26	52	19	190	36
4	80	27	75	15	295	41

** Number of fungi in $1/10^4$ gm. of soil; ** number of bacteria in $1/10^6$ gm. of soil; each is the average count on five plates.*

day after treatment the number of organisms was about equal in the rhizospheres of the frozen and healthy seedlings, but, as time went on, a greater difference in numbers became apparent, especially in the case of the bacteria, but there was a gradual decrease in numbers in the rhizosphere of the healthy seedlings, the reason for which is not immediately apparent.

Table VII gives comparative figures in individual experiments of diseased, frozen, and healthy seedlings taken two and three days after treatment. In the rhizosphere of the frozen seedlings the numbers of bacteria and actinomycetes were much more numerous on the third than on the second day; thus they were 430% greater than those of the healthy seedlings on the third day, whereas they had been only 240% greater on the second day. The diseased seedlings supported a flora that was much larger than that of the healthy seedlings and slightly larger than that of the frozen ones, although the difference was not nearly so pronounced in the third day after freezing.

The results show that the death of the seedlings, accomplished artificially, induced closely approximate quantitative effects in the rhizosphere as did death caused by the pathogen. In both cases the numbers of organisms increase from day to day after necrosis has started and this increment is very great with bacteria and actinomycetes, but is only slight with fungi. Thus it is apparent that the utilization of necrotic tissue and substances released in this breakdown probably contributes substantially to the marked increase in the number of microorganisms in the rhizosphere of diseased seedlings. The death of beet seedlings in sandy loam soil caused by *P. aphanidermatum* produced a quantitative rhizosphere response comparable to that produced by seedlings killed in Brookston clay loam by *P. ultimum* and *A. cochlioides* and this would be expected if the necrotic tissue, which in both cases is produced so soon after infection, is responsible for this rhizosphere effect.

TABLE VII

A COMPARISON IN THE NUMBER OF FUNGI, BACTERIA, AND ACTINOMYCETES IN THE RHIZOSPHERE OF HEALTHY, DISEASED, AND FROZEN SEEDLINGS GROWN IN EXETER SANDY LOAM, AT TWO AND THREE DAYS AFTER FREEZING

Days after treatment	Fungi on:						Bacteria and actinomycetes		
	Peptone–glucose agar			Czapek's agar					
	Diseased	Frozen	Healthy	Diseased	Frozen	Healthy	Diseased	Frozen	Healthy
2	41*	71	15	23	19	17	333**	159	92
2	11	15	13	12	14	12	90	91	40
2	30	15	12	50	18	12	371	148	34
Av.	27	34	13	28	17	14	265	133	55
3	15	19	11	16	23	14	86	217	37
3	61	49	30	75	55	23	746	458	63
3	76	33	44	76	69	48	528	370	136
Av.	51	34	28	56	49	28	453	348	79

** Number of fungi in $1/10^4$ gm. of soil; ** number of bacteria and actinomycetes in $1/10^6$ gm. of soil; average count of five plates in each.*

Control of Sugar Beet Rootrot

Since *Phoma betae* is not present on sugar beet seed that is used commercially in southern Ontario and, since nontreated seed, planted in steam sterilized soil, gives healthy stands of seedlings, rootrot control in this district resolves itself into one of controlling soil-borne pathogens.

It has been demonstrated that the turning in of green cover crops is very effective in changing the microbiological balance of soils. Hildebrand and West (8) under their experimental conditions reported that soybeans changed the flora to such an extent that strawberry rootrot was almost prevented and at the same time *Thielaviopsis basicola*, which is a parasite on tobacco, became much more abundant. Green rye has been reported likewise to be quite effective in a similar way in reducing the amount of scab that occurs on potatoes (14).

In an experiment two successive crops of soybeans were grown in 'rootrot' Brookston clay loam soil, and on Aug. 6 and Nov. 19, 1946, the soybeans at their maximum succulence were ground finely and incorporated with the soil in which they had grown. A similar amount of Brookston clay loam soil was untreated and both lots were kept in the greenhouse throughout the course of the experiment. Sugar beet seed was planted in each on May 26, 1947.

In the soybean treated soil the final stand was increased from 18 to 49% and, in addition, the plants were much larger (Fig. 34). It would appear that here too the soybean residue had reduced disease incidence through the shift in the microbiological balance that it must have occasioned in the experimental

soil. It is equally apparent, however, from the increased vigor of the seedlings, that other factors are operating as well and these may not be without significance in relation to the reaction of the host to the parasites.

The efficacy of two antibiotics, penicillin and streptomycin, applied as seed treatments, was tested. Preliminary tests showed that penicillin thus applied was ineffective and further investigation was carried on only with streptomycin. A stock solution of streptomycin was made by adding 10 cc. of water at a pH of 3 to 1 gm. (80,000 units) of crystalline streptomycin. Fifty seeds were soaked in each of various concentrations of this solution for 48 hr. at 5° C. previous to planting in naturally-infested Brookston clay loam soil.

The results summarized in Table VIII show that streptomycin definitely does control rootrot and that there is a wide latitude between effective and phytotoxic concentrations. A higher percentage of emergence might have

TABLE VIII

EFFECT OF SEED TREATMENT WITH STREPTOMYCIN ON INCIDENCE OF ROOTROT IN SUGAR BEETS GROWN IN NATURALLY-INFESTED BROOKSTON CLAY LOAM SOIL

Treatment	Germination, %	Rootrot, %
Stock solution	66	0.0*
" " ½ strength	64	0.0
" " 1/50 strength	72	22.2
Untreated	54	74.0

** 6% of the seedlings showed toxic effects.*

resulted if the seed had been treated at different temperatures or at a higher pH. Further tests should be conducted with both purified and impure streptomycin since, if the antibiotic were effective in its crude state, it might be economically feasible to use it commercially for seed treatments. This experiment demonstrates, however, that an antibiotic that is produced by the soil organism, *Streptomyces griseus*, can be used effectively as a seed treatment that prevents the parasitic activity of soil pathogens. This suggests the desirability of extensive studies of the potential significance of antibiotic substances as fungicides. Such antibiotic substances may well be produced by fungi and bacteria in large numbers and in a great variety of chemical patterns, and it is quite possible that they might be used effectively and economically as protective or eradicant fungicides. The possibility is all the more worthy of attention since the results have, in general, been so disappointing in the attempts to control soil-borne parasites through artificially induced shifts in the microbiological balance of infested soils.

Throughout this study it was repeatedly observed that commercial fertilizers may aid in reducing the amount of rootrot. In experiments designed to investigate their effect, however, fertilizer had no influence on pre-emergence

blight but did reduce postemergence blight considerably (Table IX). The beet was found to pass through its primary development much more quickly when grown in soil to which fertilizer had been added. It is felt that, in as

TABLE IX

EFFECT OF THE ADDITION OF COMMERCIAL FERTILIZER ON EMERGENCE AND INCIDENCE OF ROOTROT IN SUGAR BEETS GROWN IN BROOKSTON CLAY LOAM

Treatment	Emergence, %	Rootrot, %	Final stand, %
Fertilizer*	82.3	17.0	68.5
Check	84.6	55.8	37.6

** 2-16-8 Fertilizer was applied at the rate of 400 lb. per acre.*

much as this significantly reduces the time the seedlings are susceptible to *Pythium*, this is the most probable explanation of the action of fertilizers, but other possibilities have not been explored.

Hildebrand *et al.* (7) found that Arasan was very effective in controlling rootrot of sugar beets and this paper presents some preliminary experiments carried on to investigate the mechanism of action of Arasan in preventing root deterioration. Its direct action was tested by placing a small lump of Arasan in the center of a number of Petri dishes that contained potato or cornmeal extract agar. In each dish one of the pathogens was inoculated at the side and allowed to grow toward the Arasan. Growth of *A. cochlioides* was retarded initially at a distance of one inch, but after a few days it usually grew to within one-half inch of the Arasan (Fig. 35).

P. aphanidermatum was much retarded, but not inhibited, as it approached the Arasan (Fig. 36) and finally grew very close to it. *P. ultimum*, *R. solani*, and *Penicillium* sp. reacted in a similar way. The distance at which retardation occurred was slightly different on the two media and in the soil the reaction may be quite different. A direct fungicidal component, however, seems to account in part at least for the effectiveness of Arasan.

The method by which, in these experiments, Arasan acted in advance was not known and to test the possibility of the toxin being volatile, a thin slice of media was removed between the lump of Arasan and the pathogen. In all cases, however, this space eliminated all retardation by Arasan and thus the toxin must have been diffusing through the media.

The striking effectiveness of soil applications suggested the possibility that Arasan might be acting in part through some disturbance of the microbiological balance in the soil unfavorable to the pathogens. Accordingly, some experiments were run to find out what influence this compound had on the soil flora. Arasan at the rate of 0.07 gm. per 27.5 cu. in. air-dried soil was uniformly mixed with Brookston clay loam. This is equivalent to an application of 4 lb. per acre (7), the rate at which it is now used commercially. Then

125 cc. of water was added to this and to the untreated soil used in the check and both were kept in open flasks. At weekly intervals dilution plates were poured as described previously and counts of bacteria and fungi were taken and the experiment was replicated.

These results, which are given in Table X, show that the number of fungi in the Arasan treated soil was reduced within two days to about one-fourth of that in the check soil and that this relationship persisted even after 67 days.

TABLE X

NUMBER OF FUNGI AND BACTERIA AFTER DIFFERENT INTERVALS IN ARASAN-TREATED AND NONTREATED BROOKSTON CLAY LOAM

Days after treatment	Fungi on:		Bacteria and actionomycetes	
	Treated	Untreated	Treated	Untreated
2	1.0*	11.5	58**	42
8	2.5	5.5	80	39
15	1.5	5.5	76	19
21	0.5	4.5	55	15
29	2.0	6.0	36	21
36	2.0	10.0	31	17
67	1.0	6.5	18	17

* *Number of fungi in $1/10^4$ gm. of soil.*

** *Number of bacteria and actinomycetes in $1/10^6$ gm. of soil; each is the average count on eight plates.*

In the case of the bacteria, on the contrary, the Arasan treatment stimulated development initially, the number reaching a maximum after about 15 days. By the 67th day the numbers had returned to normal. These results are highly suggestive and indicate the desirability of carrying this phase of the investigation further.

Discussion and Summary

Of the four rootrot pathogens of sugar beets that were found in southern Ontario, *A. cochlioides*, which causes what is commonly called blackroot of sugar beets, is easily the most important economically. That it was not previously reported in Canada probably is due to the difficulty of isolating it by ordinary laboratory techniques. However, by the single expedient of immersing in water bits of infected tissues, not only is the fungus induced to emerge, but so also are the other three pathogens that were encountered. Even *R. solani* grows extremely well under these conditions as, unfortunately, do so many saprophytes, such as species of *Actinomyces*, *Penicillium*, and *Fusarium*. In very young infections, however, frequently only the pathogenic fungi were observed and in all cases the development of most bacteria was greatly retarded in the water and their effects on the fungi largely neutralized. This method is not only very convenient but also saves a great deal of time, inasmuch as the four pathogens can be readily identified within a few hours after the infected tissues are placed in water.

If seedlings become infected with *A. cochlioides* before they are about two weeks old, they soon die, whereas, if they are attacked at later stages, they may live for a long time and may even recover. Seedlings recover only if the air and soil moisture becomes limiting and the temperature is relatively low. In such studies as have been made of the physiology of *A. cochlioides* the most striking feature has been its marked sensitivity to relative humidities only slightly below saturation. Growth of this fungus was inhibited by a humidity of 98.7%, determined by the sulphuric acid method. Retardation of growth was detected even in a 0.2 *M* sucrose solution and this is equivalent to a relative humidity of 99.8%. This organism cannot survive in a sucrose solution that has an osmotic pressure of 15.6 atmospheres. *A. cochlioides* appears to be more sensitive to humidity than many of the pathogenic bacteria (4 and 15) and the fungi tested by Hawkins (6). Considering this sensitivity, humidity may be an important limiting factor in the parasitic activity of *A. cochlioides*. The intercellular humidity of the sugar beet was not measured, but the humidity of intercellular spaces in plants is quite variable. Thut (17) has reported that the intercellular humidity in unwilted leaves in some plants may be as low as 90%.

Whether penetration of *A. cochlioides* is direct, stomatal, or associated with wounds has not been definitely determined. Kendrick (10) reported that the closely related species *Aphanomyces raphani* entered the radish only through natural wounds made by the emergence of secondary roots. *A. cochlioides* must have some other portal of entry, because it infects the beet before secondary roots are produced. The fact that infection occurs in the hypocotyl, which has a thick cuticle and numerous open stomata, suggests strongly that infection may take place through the stomata. The intercellular locus of the mycelium would tend to support this view. This fungus invades only the cortical tissue of the seedling, the hypocotyl being the vulnerable point of attack, but the fungus may grow down into the cortical tissue of the root or may invade the root cortex after this has begun to slough off.

In southern Ontario the chronic phase of attack has been conspicuously lacking and even in fields in which it was most severe, has not been found to affect more than 0.1% of the beets. This virtual absence of the chronic phase contrasts sharply with the condition reported by Coons *et al.* (3) in the northeastern sections of the United States. Here the chronic effects of *A. cochlioides* are particularly serious.

Buchholtz and Meredith (2) found a marked difference in the ability of six isolates to produce the chronic phase and two of the isolates caused no rotting whatsoever. This indicates that there are strains of *A. cochlioides* whose infection capabilities vary. Such strains would seem to be more abundant in the northeastern part of the United States than in Ontario since no strain differences were obtained throughout these investigations. On the other hand, some vector may be necessary for the initiation of the chronic phase. This is suggested at least in the results of Buchholtz and Meredith (2) who

only observed typical rootrot in an extremely low percentage of beets that were unwounded, even when they used isolates that rotted all the beets when they were wounded. This possibility is worthy of further investigation.

The *Pythium* spp. are intracellular parasites and only attack the seedling during primary development. Resistance that begins simultaneously with secondary growth may be either mechanical or physiological, but is probably the latter.

The four pathogens were found only in the top soil and *A. cochlioides* and *P. ultimum* were most abundant in the two inches of soil at the surface. The distribution of the causal organisms was closely correlated with soil type, *A. cochlioides* being limited to the clay soil and *P. aphanidermatum* to the sandy loam soil. Some evidence was obtained that suggested that the explanation was probably microbiological.

The flora in the rhizosphere of the diseased seedlings was much greater, especially in bacteria and actinomycetes, than in the rhizosphere of the healthy seedlings. A comparable quantitative increase in microorganisms resulted when the seedlings were killed artificially with liquid carbon dioxide, which suggested that the utilization of necrotic tissue probably contributes substantially to the noticeable increase in the flora.

Streptomycin, which is produced by *Streptomyces griseus*, was very effective as a seed treatment and prevented rootrot by soil pathogens. The potential significance of antibiotic substances and the possibility that they might be used effectively and economically as protective or eradicant fungicides is stressed.

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STUDIES IN FOREST PATHOLOGY

VII. DECAY IN WESTERN HEMLOCK AND FIR IN THE FRANKLIN RIVER AREA, BRITISH COLUMBIA[1]

By D. C. Buckland,[2] R. E. Foster,[3] and V. J. Nordin[4]

Abstract

An investigation of decay in western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and fir (mainly *Abies amabilis* (Loud.) Forb.) in the Juan de Fuca forest region of British Columbia has shown that the major organisms causing root and butt rots are the same in both species. These are *Poria subacida* (Peck) Sacc., *Fomes annosus* (Fr.) Cke., *Armillaria mellea* Vahl ex Fr., *Polyporus sulphureus* Bull. ex Fr., and *P. circinatus* Fr. Those organisms causing trunk rots of western hemlock, in decreasing order of importance, are *Fomes pinicola* (Sw.) Cke., *F. Pini* (Thore) Lloyd, *Stereum abietinum* Pers., *Fomes Hartigii* (Allesch.) Sacc. and Trav., and *Hydnum* sp. (*H. abietis*). These same organisms causing trunk rots of fir, in decreasing order of importance, are *Fomes pinicola*, *Stereum abietinum*, *Hydnum* sp. (*H. abietis*), *Fomes Pini*, and *Fomes Hartigii*. The logs of 963 western hemlock were analyzed in detail. Maximum periodic volume increment was reached between 225 and 275 years of age. Maximum periodic volume increment was reached between 275 and 325 years of age in the 719 fir that were analyzed. Scars were the most frequent avenue of entrance for infection. In 59% of the cases of infection studied the fungus had entered through wounds.

Introduction

In 1945 a request was made by logging interests in the Juan de Fuca forest region that information be obtained on the decays of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and fir (mainly *Abies amabilis* (Loud.) Forb.). Basic knowledge of the decay loss in stands of various ages was particularly desired for working out cutting schedules in a sustained yield forestry program. To obtain the information needed for the efficient utilization of the 500,000 acres in question, it was necessary that precise inventories of the region be made. The accuracy of forest inventories depends largely on the accuracy with which the expected loss through decay can be predicted. It was the main object of this study, therefore, to determine as closely as possible the loss through decay in relation to age and diameter in western hemlock and fir. Wide variations in the cause and extent of decay in a given host are known to exist in different regions. It was not possible, therefore, to use the results of previous investigations (3, 4, 6, 11) to answer the request. The field work was carried out in 1945 and 1946 and the information obtained is presented in this paper.

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Location of Studies

It has been demonstrated repeatedly that wide variation in the growth of a tree species and in the cause and extent of decay occurring in it may exist between regions. Therefore the field work in this study was confined to one region containing as nearly as possible a uniform forest type. The study was carried out mainly in the Franklin River area through to the Nitinat River area, in the northwestern portion of the Juan de Fuca forest region (Text-fig. 1) on southern Vancouver Island, B.C.

Thirteen areas containing 26.1 acres were analyzed. These study areas were situated in four valleys or centers as indicated in Text-fig. 1. The first, a valley running from the headwaters of the Sarita River to Coleman Creek, contained 13.0 acres in six study areas. The second, a valley running northeast of Darlington Lake, contained 7.5 acres in four study areas. The third, in the Francis Lake valley, contained 1.6 acres in one study area. The fourth, located south of the Nitinat River, contained 4.0 acres in two study areas.

Forest Type

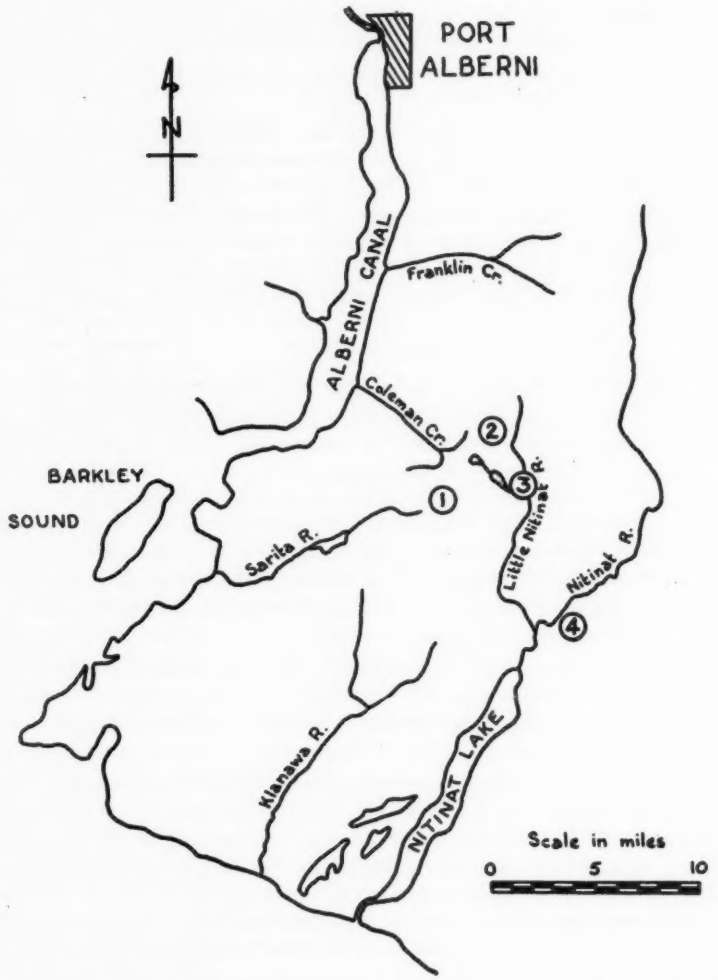
The Juan de Fuca forest region contains heavy stands of coniferous timber. The region is mountainous and the heavy stands reach to high elevations with few rock outcroppings. According to Silburn (10), 96% of the merchantable timber and all the immature timber are accessible. Western hemlock and fir form the greatest portion of these stands, but a considerable amount of western red cedar (*Thuja plicata* D. Don.) and Douglas fir (*Pseudotsuga taxifolia* (Poir.) Britt.) is also present. Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and western white pine (*Pinus monticola* Dougl. ex D. Don.) occur scattered throughout the region, with yellow cedar (*Chamaecyparis nootkatensis* (Lamb.) Sudw.) at higher elevations.

The four study areas analyzed, indicated in Text-fig. 1, contained an average gross volume of 45,900 bd. ft. of merchantable timber per acre. Each area, based on geographic location, contained a slightly different forest type as follows:

1. The stands were mainly western hemlock and fir, with western red cedar occurring frequently. In the valley bottom and on the slopes, the average gross merchantable volume per acre in mature stands was 42,000 bd. ft., dropping to 41,300 bd. ft. on the hill tops. In the young stands western hemlock predominated with a considerable volume of true fir. In the overmature stands western hemlock and red cedar were the only merchantable trees, with a thrifty stand of small fir of nonmerchantable pole size frequently observed.

2. Western hemlock and fir predominated in the valley bottom, but at higher elevations fir was not present, and yellow and red cedar became frequent. Gross merchantable volumes averaging 59,600 bd. ft. for mature stands of western hemlock and fir were found in the valley bottoms, dropping to 40,100 on the hill tops.

3. There was less fir here than in the previous types, but Douglas fir was present. The western hemlock and fir together contained a volume of 49,000 bd. ft. gross merchantable volume per acre.



TEXT-FIG. 1. *Location of the study areas in the Juan de Fuca forest region. The numbered circles indicate the approximate location of the areas studied.*

4. The stands here were made up chiefly of western hemlock and red cedar, with fir rarely present. The former species ran to an average volume of 58,200 bd. ft. per acre gross merchantable volume in mature stands.

Method of Study

Sample Plots

In 1945 the plots had contained 0.1 acre each, but laying out these plots and analyzing the data obtained was too time consuming for the small areas. Therefore, each group of four adjacent plots was analyzed as one and in 1946 sample plots two chains square, containing 0.4 acre, were laid out mechanically in lines four chains apart. This procedure was varied somewhat to avoid concentrations of species other than western hemlock and fir.

The majority of these plots were established in felled and bucked timber. Where samples were required of a forest area outside felled and bucked timber, the trees were felled and bucked according to the standard practice for the region. To obtain age–diameter relationships and other data, all trees were felled on each sample plot regardless of merchantability.

Field Procedure

All trees on the sample plots were scaled according to the British Columbia Log Rule (9). Each tree was scaled as bucked for merchantable volumes according to the practice of the logging company operating on the area, and to a 10-in. top diameter for standardization of the results. Stump height, diameter at stump height inside bark, diameter at breast height inside and outside bark, and wherever possible, total height of the bole, were recorded for each tree. Notes were kept concerning the position of crooks, large knots, spiral grain, conks, and other visible defects. The location and size of decayed areas were recorded and volume deductions calculated according to standard practice. All data were recorded on U.S. Forest Service form 228A, giving a clear picture of the position and extent of all defects and abnormalities.

The age of each tree was determined at stump height and total age computed by adding to this figure the estimated number of years required for a tree to grow to stump height. This latter was determined by sectioning western hemlock and fir reproduction in adjacent areas. The diameter increment was recorded for each tree by counting the number of rings per inch along the average radius of the stump. Where decay or other defects in the butt made a ring count impossible, the number of growth rings of the destroyed portion was considered the same as that for adjacent trees showing a similar increment pattern.

Compilation of Data

A clear picture of each tree sampled with the position and extent of all defects or abnormalities was given by the forms used in the field. Cubic volumes can be obtained from these forms with a planimeter but they have not been given in compiling the results of this study, as there is no standard practice for their use in British Columbia at the present time. Compilation was made of the merchantable board foot volumes (or volumes utilized

according to present practice) and volumes to a standard 10-in. top diameter inside bark unless otherwise stated. All diameter breast height measurements are outside bark.

Results

Fungi Causing Decay in Living Trees

Occurrence of Fungi Causing Decay

The major decay organisms were approximately of the same relative frequency in both western hemlock and fir as shown in Table I. The well known

TABLE I

The occurrence of fungi causing decay in living western hemlock and fir in the Franklin River area

Organism	Type of decay	Western hemlock		Fir	
		Number of infections	Percentage of all infections	Number of infections	Percentage of all infections
Root and butt rots		259	51.2	83	41.9
Poria subacida	White spongy	104	20.6	35	17.7
Fomes annosus	White spongy	35	6.9	6	3.0
Armillaria mellea	Yellow spongy	71	14.0	13	6.6
Polyporus sulphureus	Brown cubical	9	1.8	5	2.5
Polyporus circinatus	White pitted	7	1.4	2	1.0
Polyporus Schweinitzii	Brown cubical			1	0.5
Poria Weirii	Brown stringy	11	2.2		
Poria nigrescens		1	0.2		
Poria asiatica	Brown cubical	1	0.2		
Mixed and unknown		20	3.9	21	10.6
Trunk rots		227	44.9	105	53.0
Fomes pinicola	Brown cubical	101	20.0	39	19.7
Fomes Pini	White pitted	46	9.1	2	1.0
Stereum abietinum	Brown cubical	29	5.7	30	15.1
Fomes Hartigii	White spongy	13	2.6	2	1.0
Hydnum sp. (*H. abietis*)	Yellow pitted	7	1.4	11	5.6
Fomes applanatus	White spongy	7	1.4		
Polyporus abietinus	White spongy			3	1.5
Mixed and unknown		24	4.7	18	9.1
Sap rots		20	3.9	10	5.1
Fomes pinicola	Brown cubical	12	2.3	5	2.5
Polyporus abietinus	White spongy pitted	6	1.2	4	2.1
Fomes applanatus	White spongy	1	0.2		
Fomes Hartigii	White spongy			1	0.5
Mixed and unknown		1	0.2		
Total		506		198	

root and butt rots caused by *Poria subacida* (Peck) Sacc., *Fomes annosus* (Fr.) Cke. (Plate I), and *Armillaria mellea* Vahl ex Fr. (Plate II), and the trunk rot, caused by *Fomes pinicola* (Sw.) Cke. (Plate III), occurred frequently

in both species. A major trunk rot, caused by *Fomes Pini* (Thore) Lloyd (Plate VI), showed a marked difference in occurrence on western hemlock and fir. Less than 1% of all infections, or two cases of decay, caused by *F. pini* were found on fir in the sample plots investigated. *Polyporus Schweinitzii* Fr., the cause of a brown cubical butt rot of frequent occurrence in many tree species, did not occur on western hemlock on the study area, and was found only once on fir.

The frequent occurrence of *Stereum abietinum* Pers. (Plate IV), causing a brown cubical pocket rot of both western hemlock and fir, is of much interest. This fungus has not been reported previously as an important decay organism of either tree species. *Hydnum* sp. (*H. abietis*) (7) occurred so frequently in living western hemlock and fir that it can be classified as the cause of a major decay of both species. This is the same fungus as that referred to as *Hydnum abietis* Hubert by Englerth (6), who reported it as the cause of a minor decay in western hemlock.

Poria nigrescens Bres., reported as causing decay in living Sitka spruce (2), was found in living western hemlock but not in fir. *Poria asiatica* (Pilât) Overh., reported as an important decay of western red cedar (5), was also found in western hemlock but not in fir.

Ganoderma oregonense Murr., reported by Englerth (6) as causing decay of western hemlock, was observed on this species in the area under investigation, but did not occur on any of the sample plots. *Echinodontium tinctorium* Ell. and Ev., reported on western hemlock and fir by Bier (1, 3), Englerth (6), and others, was not observed anywhere in the region.

Avenues of Entrance of Infections

As the avenues of entrance of infections might on occasion be used as an external indication of decay, they are worthy of attention. During the course of this study there were 338 cases where these entrances were noted. These were all either roots, scars, branch stubs, or broken or dead tops. Table II lists the organisms responsible for the decays noted in this study and their place of entrance.

It is interesting to observe that of the 338 infections where the avenue of entrance was recorded, 198, or approximately 60%, entered the trees through scars. As would be expected, a great number of the root and butt rots entered through the roots, but an even greater number of this group entered through scars. Of the trunk rots the majority, 58%, entered through scars and 36% through branch stubs. These figures stress the great number of infections that gain entrance through scars. As the majority of these scars were caused by falling trees or snags, the danger of partial or selective logging in hemlock–fir stands is indicated.

Relative Importance of Fungi Causing Decay

Comparison of the relative importance of the fungi causing decay in living western hemlock and fir is shown in Table III. This table shows only those

TABLE II

AVENUES OF ENTRANCE OF INFECTIONS IN WESTERN HEMLOCK AND FIR IN THE FRANKLIN RIVER AREA

(BASED ON 338 CASES OF INFECTION)

Organism	Total number of infections	Avenues of entrance			
		Roots	Scars	Branch stubs	Damaged tops
		Number of infections			
Root and butt rots	168	74	91	3	
Poria subacida	79	31	46	2	
Fomes annosus	13	6	7		
Armillaria mellea	56	32	24		
Polyporus sulphureus	5	1	3	1	
Polyporus circinatus	9		9		
Poria Weirii	5	3	2		
Poria asiatica	1	1			
Trunk rots	119	2	70	43	4
Fomes pinicola	45	2	29	12	2
Fomes Pini	33		21	12	
Stereum abietinum	21		10	11	
Fomes Hartigii	7		1	5	1
Hydnum sp. (H. abietis)	5		3	2	
Fomes applanatus	6		4	1	1
Polyporus abietinus	2		2		
Sap rots	20		19	1	
Fomes pinicola	15		14	1	
Polyporus abietinus	3		3		
Fomes applanatus	1		1		
Fomes Hartigii	1		1		
Mixed and unknown	31	13	18		
TOTALS	338	89	198	47	4

organisms responsible for more than 5% of the total volume of wood lost through decay in either species. While the eight major decay organisms listed in Table III were not of the same relative importance in both species of trees, the percentage of the total volume of decay caused by these organisms, over 80%, was almost identical. These decays, together with mixed and unknown rots, accounted for 90% of all volume loss from decay.

The root and butt rots (Plates I and II), while responsible for decay in approximately 50% of all infected trees (Table I), were only responsible for approximately 25% of the volume loss from decay (Table III). This indicates that these rots are restricted primarily to a few feet in the butt, while the trunk rots (Plates III to VII) frequently grow considerable distances through the bole of the infected tree.

Fomes pinicola (Plate III), causing 40% of the decay in both species, appears to be responsible for the major loss in both western hemlock and fir.

TABLE III

THE RELATIVE IMPORTANCE OF FUNGI CAUSING THE MAJOR LOSS THROUGH DECAY IN WESTERN HEMLOCK AND FIR IN THE FRANKLIN RIVER AREA

Organism	Volume of decay			
	Western hemlock		Fir	
	Percentage of total infections of all trees	Percentage of total volume of decay	Percentage of total infections of all trees	Percentage of total volume of decay
Root and butt rots				
Poria subacida	1.4	10.2	0.8	11.5
Fomes annosus	1.0	7.3	Trace*	Trace
Armillaria mellea	0.9	6.4	0.3	4.7
Polyporus sulphureus	0.1	0.8	0.4	5.7
Totals	3.4	24.7	1.5	21.9
Trunk rots				
Fomes pinicola	5.8	40.8	2.9	40.5
Fomes Pini	1.8	12.8	Trace	1.3
Stereum abietinum	0.4	3.0	0.4	6.2
Hydnum sp. (H. abietis)	0.2	1.3	0.9	12.1
Totals	8.2	57.9	4.2	60.1
TOTALS	11.6	82.6	5.7	82.0

* *"Trace" indicates that the percentage of rot was less than 0.1.*

Poria subacida (Plate I), *Armillaria mellea* (Plate II), and *Stereum abietinum* (Plate IV) caused similar damage in both species, while the other organisms varied appreciably in relative importance. *Fomes annosus* (Plate I) and *Fomes Pini* (Plate VI), while occurring to a very limited degree in fir, were of importance in western hemlock. The converse was true, however, in the case of *Polyporus sulphureus* Bull. ex Fr. (Plate III) and *Hydnum sp. (H. abietis)* (Plate V). While there are several instances of different relative importance of specific decays in the two species of trees, the whole decay picture for both western hemlock and fir is remarkably similar.

DECAY IN RELATION TO AGE

Rather than depicting the condition of a stand at each period of its life, the results of this study give a picture of the condition of each tree of a given age in the stand. In computing maximum net periodic increment correctly, the life cycles of the trees on a single area throughout a complete rotation should be observed. It is evident, however, that such a method is not practicable. The only feasible method known at present of obtaining data to work out a relationship between decay and age in uneven-aged mature stands is to

study individual trees. The use of such a basis in the mature and over-mature stands investigated gives erroneous results as the trees that did not survive early competition and disease, recent losses being indicated by the presence of snags, are of necessity omitted. It can also be seen that this method of carrying out a study on the individual tree basis will give too high a maximum net periodic increment.

Incidence of Decay

It is indicated in Table IV, which outlines the relation between age and incidence of decay in western hemlock and fir in the Franklin River area,

TABLE IV

THE RELATION BETWEEN AGE AND INCIDENCE OF DECAY IN WESTERN HEMLOCK AND FIR IN THE FRANKLIN RIVER AREA

Age class	Percentage of trees with decay	
	Western hemlock	Fir
51 – 100	8.2	0.0
101 – 150	23.9	11.1
151 – 200	30.9	15.1
201 – 250	36.7	27.0
251 – 300	44.2	28.8
301 – 350	58.6	47.6
351 – 400	68.8	88.9
401 – 450	85.1	100.0
451 – 500	86.0	100.0

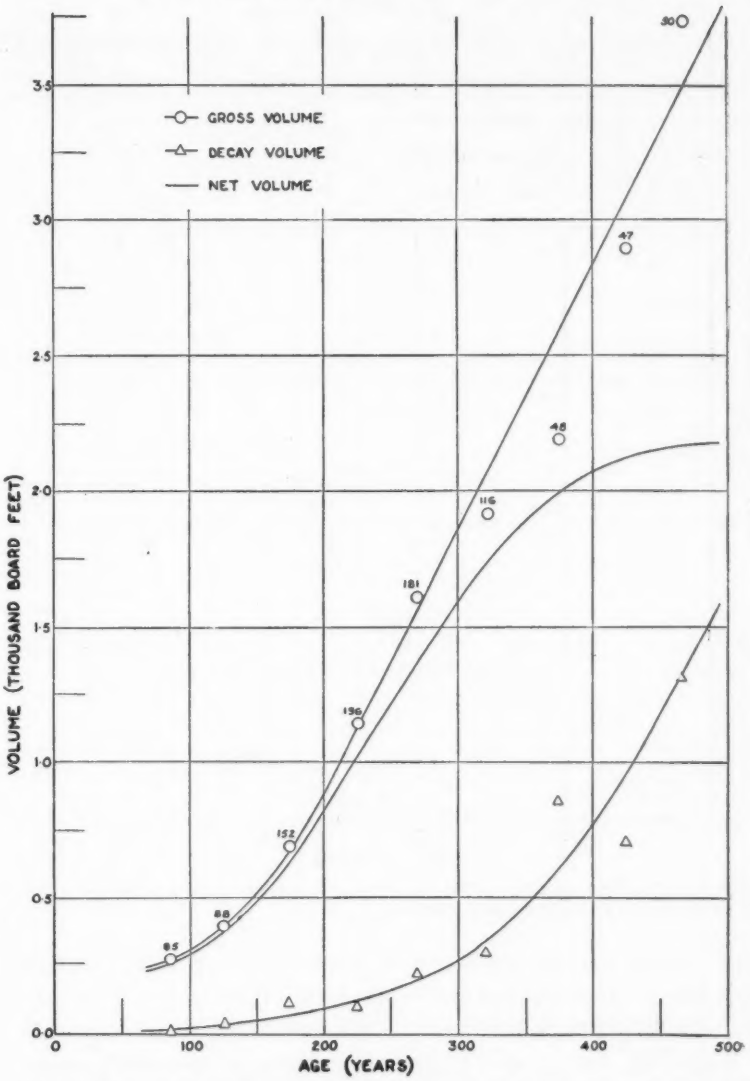
that fir is a shorter-lived tree than is western hemlock. While all fir over 400 years of age were infected, it was found that this condition was not true of western hemlock in the 500-year age group covered by the study.

It is evident from Table IV that (up to 350 years of age) fir is more resistant to infection than is western hemlock. The resin pockets present in the bark of the former may account for this to some extent. The resin forms a coating that probably prevents the entrance of decay organisms through scars and wounds, the main avenue of entrance for wood-rotting fungi (Table II).

Losses Through Decay

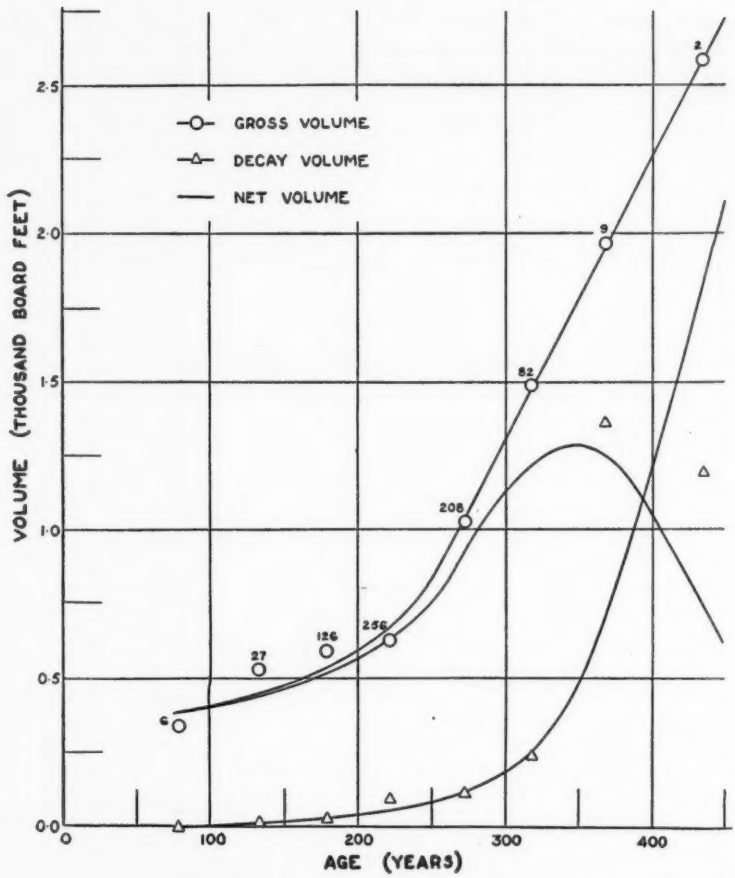
The average gross board-foot volume, the volume of decay, and the average net volume on age for western hemlock and fir are shown in Text-figs. 2 and 3 respectively. The curved values computed from these graphs are given in Tables V and VI.

Western hemlock (Text-fig. 2 and Table V) reached a maximum gross periodic increment of 490 bd. ft. per tree from 225 to 275 years of age. This increment did not vary in the 50-year periods from 275 to 525 years. During the 225- to 275-year period the maximum net periodic increment of 400 bd. ft.



TEXT-FIG. 2. *Relationship between age and gross volume, decay volume, and net volume, to a 10-in. top, in western hemlock in the Franklin River area.*

was also reached with a decay loss of 13.0%. For the 50-year periods from 175 to 325 years, the net periodic increment was within 50 bd. ft. of the maximum. Following the peak net growth (225 to 275 years of age), the net periodic increment decreased to 60 bd. ft. for the period from 475 to 525



TEXT-FIG. 3. *Relationship between age and gross volume, decay volume, and net volume, to a 10-in. top, in fir in the Franklin River area.*

years. As the net periodic increment never became a negative quantity, the average net volume gained continually, being 230 bd. ft. per tree at 75 years of age and increasing to 2180 bd. ft. at 475 years of age.

Fir (Text-fig. 3 and Table VI) reached a maximum gross periodic increment of 480 bd. ft. per tree from 275 to 325 years of age. As with western hemlock, this increment did not vary throughout the remainder of the periods studied. The maximum net periodic increment of 310 bd. ft. was reached during the same period as the gross increment. While the 50-year period preceding this peak showed an increment of 300 bd. ft., the period following it showed a loss of 30 bd. ft. net. This sudden drop is further emphasized in the average

TABLE V

THE RELATIONSHIP BETWEEN AGE AND VOLUME OF DECAY AND NET PERIODIC INCREMENT IN WESTERN HEMLOCK IN THE FRANKLIN RIVER AREA

Age	Number of trees	Average gross volume, bd. ft.	Average decay volume, bd. ft.	Percentage of decay	Average net volume, bd. ft.	Average gross periodic increment, bd. ft.	Average net periodic increment, bd. ft.
75	85	240	10	4.2	230		
						160	140
125	88	400	30	7.5	370		
						290	260
175	152	690	60	8.7	630		
						430	370
225	196	1120	120	10.7	1000		
						490	400
275	181	1610	210	13.0	1400		
						490	350
325	116	2100	350	16.7	1750		
						490	240
375	48	2590	600	23.2	1990		
						490	130
425	47	3080	960	31.2	2120		
						490	60
475	50	3570	1390	38.9	2180		

TABLE VI

THE RELATIONSHIP BETWEEN AGE AND VOLUME OF DECAY AND NET PERIODIC INCREMENT IN FIR IN THE FRANKLIN RIVER AREA

Age	Number of trees	Average gross volume, bd. ft.	Average decay volume, bd. ft.	Percentage of decay	Average net volume, bd. ft.	Average gross periodic increment, bd. ft.	Average net periodic increment, bd. ft.
75	6	390	0	0.0	390		
						60	50
125	27	450	10	2.2	440		
						90	70
175	126	540	30	5.6	510		
						160	130
225	256	700	60	8.6	640		
						360	300
275	208	1060	120	11.3	940		
						480	310
325	82	1540	290	18.8	1250		
						480	−30
375	9	2020	800	39.6	1220		
						480	−380
425	2	2500	1660	66.4	840		

net volumes. Starting from 390 bd. ft. per tree at 75 years of age, fir reaches a maximum net volume of 1250 bd. ft. at 325 years of age. The next 100 years shows a decline to 840 bd. ft.

Decay in Relation to Diameter

The true value of the timber in a stand is obtained from its net volume, i.e., the gross volume minus the volume of decay and natural defects. The gross volume is obtained by cruising a sample of trees in the stand on a basis of diameter at breast height. As few of the internal decays show external indications (page 328), they must be classified as hidden defects. It would be desirable, therefore, to obtain a relationship between diameter breast height and cull for decay for the purpose of estimating net volume of stands. As decay is dependent on age rather than diameter, it is necessary that a close relationship exist between diameter and age before diameter can be used as a basis for estimating decay.

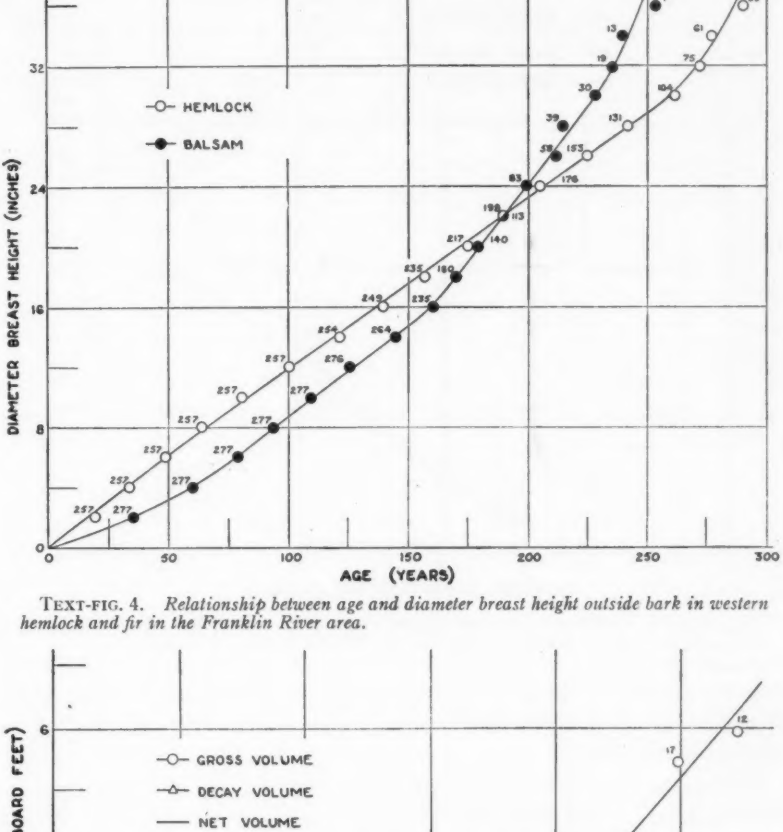
A close relationship is found to exist between age and diameter breast height for both species hemlock and fir in the Franklin River area (Text-fig. 4). The relationship of diameter to age suggests the succession of these two species in the stand. It appears that fir is the less tolerant of the two species as it suffers from suppression during its period of establishment in the stand, while western hemlock is unaffected. The growth rate of fir increases until it is equal to that of western hemlock from 60 to 160 years of age. The opening up of the stand following this period stimulates the growth of the former more than that of the latter, and at 190 years of age the two species have the same average diameter.

Western hemlock in the area shows remarkable uniformity in diameter growth over the first 250 years, reaching approximately 28 in. diameter breast height by that time. Following this the increase in diameter growth is probably indicative of stimulation of growth with some opening up of the stand.

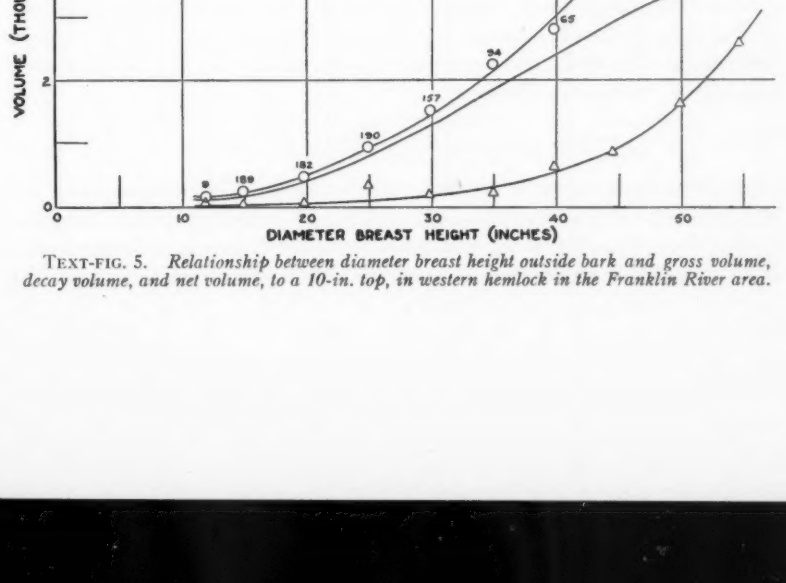
In uneven-aged stands a considerable variation in diameter at any given age is always present. It is therefore essential that any percentage deduction for decay presented in this study be applied only for a diameter class when a large sample cruise has been done. The deduction percentages would not be accurate for a small sample.

The relation between diameter breast height and volume of decay in the Franklin River area is shown in Text-figs. 5 and 6 for western hemlock and fir respectively. The curved values taken from these figures are given in Tables VII and VIII.

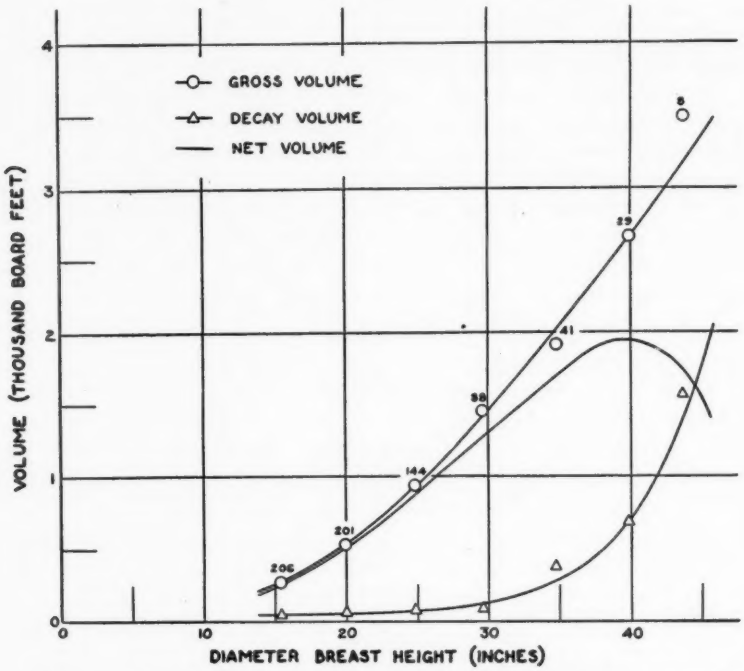
Western hemlock does not attain the maximum net volume of 3700 bd. ft. until a diameter breast height of 55 in. is reached (Table VII). At this diameter 42% of the gross volume of 6410 bd. ft. is lost through decay. The greatest net volume of growth occurs in trees between 40 and 45 in. diameter breast height. Between these two diameter limits 600 bd. ft. net volume is added. From 15 in. diameter breast height, the smallest diameter of merchantable size, up to 30 in., the percentage lost through decay varies only



TEXT-FIG. 4. *Relationship between age and diameter breast height outside bark in western hemlock and fir in the Franklin River area.*



TEXT-FIG. 5. *Relationship between diameter breast height outside bark and gross volume, decay volume, and net volume, to a 10-in. top, in western hemlock in the Franklin River area.*



TEXT-FIG. 6. *Relationship between diameter breast height outside bark and gross volume, decay volume, and net volume, to a 10-in. top, in fir in the Franklin River area.*

from 10 to 13%. Following this there is a gradual increase up to the 45-in. diameter class, after which decay destroys more volume than is gained by an increase in diameter.

Fir reaches the maximum net volume of 1900 bd. ft. at 40 in. diameter breast height. Of the 2680 gross board foot volume at this diameter, 29% is lost through decay. The greatest net volume of growth, 400 bd. ft., is added between the 25- and 30-in. diameter classes, but the net volume increase between diameters is fairly uniform from 20 to 35 in. diameter breast height, varying only 30 bd. ft. between 5-in. diameter classes. Trees of a diameter class greater than 40 in. lose considerably more volume through decay than is gained by an increase in diameter.

DECAY IN RELATION TO RATE OF GROWTH

To determine whether or not there was a relationship between decay and rate of growth, each tree was placed in a vigor group by the method described by McCallum (8). Trees that fell below an age–volume curve drawn for the whole stand were classified as slow-growing or nonvigorous and those with

TABLE VII

THE RELATION BETWEEN DIAMETER AT BREAST HEIGHT AND VOLUME OF DECAY IN WESTERN HEMLOCK IN THE FRANKLIN RIVER AREA

Diameter breast height	Number of trees	Gross volume, bd. ft.	Volume of decay, bd. ft.	Percentage of decay	Net volume, bd. ft.	Net increment between diameter classes, bd. ft.
15	189	280	30	10.7	250	
						220
20	182	530	60	11.3	470	
						340
25	190	910	100	11.0	810	
						490
30	157	1490	190	12.8	1300	
						540
35	94	2190	350	16.0	1840	
						560
40	65	2980	580	19.5	2400	
						600
45	45	3950	950	24.1	3000	
						450
50	17	5120	1670	32.6	3450	
						250
55	12	6410	2710	42.3	3700	
						0
60	2	7780	4080	52.4	3700	

TABLE VIII

THE RELATION BETWEEN DIAMETER AT BREAST HEIGHT AND VOLUME OF DECAY IN FIR IN THE FRANKLIN RIVER AREA

Diameter breast height	Number of trees	Gross volume, bd. ft.	Volume of decay, bd. ft.	Percentage of decay	Net volume, bd. ft.	Net increment between diameter classes, bd. ft.
15	206	250	10	4.0	240	
						280
20	201	540	20	3.7	520	
						370
25	144	950	60	6.3	890	
						400
30	88	1460	170	11.6	1290	
						390
35	41	2050	370	18.0	1680	
						220
40	29	2680	780	29.1	1900	
						−100
45	8	3360	1560	46.4	1800	

volumes above the curve were classified as fast-growing or vigorous. With this separation no consistent difference between the two vigor classes at a corresponding age could be found in percentage of volume lost through decay.

Losses Other Than Decay

During the course of the study, while all computations were made to a 10-in. top diameter for standardization, the actual volumes utilized by the logging company operating in the area were recorded and tabulated as indicated on page 315. Comparison of the average gross board foot volumes utilized to the average gross board foot volumes to a 10-in. top shows that in western hemlock 81% of the volume was utilized, and in fir 84%. For both species the average net volume utilized was 89% of the average net volume to a 10-in. top.

In western hemlock deductions for defects other than decay, such as breakage, crooks, shakes, and splits, in utilized volumes were 78% of those in volumes to a 10-in. top. It is evident, therefore, that as only 81% of the gross volume to a 10-in. top was utilized the majority of cull caused by defects other than decay occurs in the utilized portion of trees of this species. This is probably accounted for by the large amount of ring shake present in the butt logs of mature western hemlock.

In fir, deductions for defects other than decay in utilized volumes were only 53% of those in volumes to a 10-in. top. This would indicate that a considerable proportion of the cull to a 10-in. top for defects other than decay is for breakage.

Discussion

External indications of decay in the individual tree have, in the past, been the main basis for estimating the loss through decay in a stand. By field observations carried out during this study, it was observed that the fungus, *Fomes Pini*, was the only one that usually produced sporophores or external indications of decay. *Fomes pinicola* frequently produced fruiting bodies indicating decay, but rarely could the extent of decay caused by the fungus be estimated without examining the felled tree. As *F. Pini* caused only 12.8% of all loss through decay in western hemlock and 1.3% in fir, it is evident that decay in such areas can only be accurately estimated by a careful examination of a large sample of felled trees.

The pathological condition of one region cannot safely be assumed to be similar to that of another supporting the same species, as is shown by a comparison of the results obtained by Englerth in western Oregon and Washington (6) and those obtained in this study. Not only were some of the fungi reported by Englerth as causing major losses in decay very infrequent or entirely absent in this investigation, but also the relative losses caused by the different organisms frequently varied appreciably.

As considerable differences in loss through decay occur between two regions, it is also evident that lesser differences, although significant and important, occur between stands on different sites. An attempt was made to separate

sites in this investigation but insufficient sampling made it impossible. Extensive sampling on all sites would be required to make such an investigation complete enough for results to be utilized with accuracy in formulating management plans. A detailed site classification of the region investigated was not available at the time this study was carried out. As no significant differences in amount of decay in relation to age were observed between areas investigated, it is thought that the areas sampled were of a fairly uniform site classification.

Summary

In the northwestern portion of the Juan de Fuca forest region, between Nitinat Lake and the Alberni Canal on Vancouver Island, the logs of 963 western hemlock and 719 fir were analyzed in detail.

Decay in the former species was found to be caused by 15 organisms and in the latter by 11. The major portion of the decay loss in both hosts was caused by eight species of fungi, those producing the root and butt rots being *Poria subacida*, *Fomes annosus*, *Armillaria mellea*, and *Polyporus sulphureus*, and the trunk rots, *Fomes pinicola*, *F. pini*, *Stereum abietinum*, and *Hydnum* sp. (*H. abietis*).

Scars were the most frequent avenue of entrance for infections. In 59% of the cases of infection studied the fungi had entered through wounds.

Up to 350 years of age fir appears to be more resistant to fungus attack than western hemlock. In the latter maximum periodic volume increment was reached between 225 and 275 years of age, after which a decline occurred. In the former maximum net periodic volume increment was not reached until between 275 and 325 years of age, but following this period volume increment dropped off sharply.

A close relationship between age and diameter was found in both species. Maximum net volume of hemlock was 3700 bd. ft. to a 10-in. top at a diameter of 55 in. In fir this volume was 1900 bd. ft. at 40 in. At the peak net volume of western hemlock 42% of the gross volume was lost through decay, while in fir 29% was destroyed.

In a comparison between fast- and slow-growing trees no consistent difference was found in volume lost through decay.

In western hemlock 81% of the gross volume to a 10-in. top was utilized, and in fir 84%. Losses other than through decay were mostly top breakage in fir and ring shake in western hemlock butts.

Acknowledgments

The authors are indebted to the Forestry Department of Bloedel, Stewart, and Welch Company for continued and excellent cooperation throughout this investigation. Particular thanks are expressed to Mr. J. S. Mottishaw, Chief Forester, and Mr. E. L. Young, formerly Forester, Bloedel, Stewart, and

Welch Company, for their advice and assistance, and the services of their field parties throughout the course of the study.

Dr. J. E. Bier, Officer-in-Charge of the Dominion Laboratory of Forest Pathology in Victoria during the course of this study, contributed excellent advice and direction. Dr. Mildred K. Nobles, Associate Forest Pathologist, Ottawa, identified from numerous cultures many of the organisms responsible for the decays, rendering an invaluable service in this investigation.

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EXPLANATION OF PLATES

PLATE I

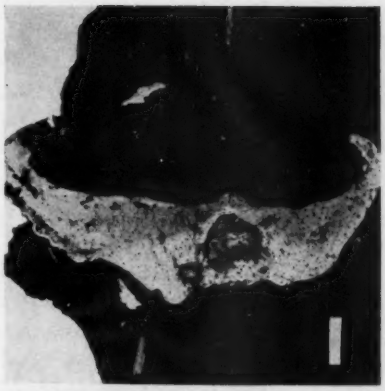
Fomes annosus and Poria subacida in living western hemlock and fir.

FIG. 1. *Fruiting body of Fomes annosus on fir.*
FIG. 2. *Spongy butt rot caused by F. annosus in western hemlock.*
FIG. 3. *Spongy butt rot caused by F. annosus in fir.*
FIG. 4. *Fruiting body of Poria subacida, cracked on drying, on western hemlock.*
FIG. 5. *Spongy butt rot caused by P. subacida in western hemlock.*

PLATE II

Armillaria mellea, Polyporus circinatus, and Poria Weirii in living western hemlock and fir.

FIG. 1. *Fruiting bodies of Armillaria mellea growing from an infected fir stump.*
FIG. 2. *Spongy butt rot caused by A. mellea in western hemlock.*
FIG. 3. *Spongy butt rot caused by A. mellea with black aerial rhizomorphs in fir.*
FIG. 4. *Fruiting body of Polyporus circinatus growing from an infected western hemlock stump.*
FIG. 5. *White pitted butt rot caused by P. circinatus in western hemlock.*
FIG. 6. *Decay caused by Poria Weirii in western hemlock.*
FIG. 7. *Laminate butt rot caused by P. Weirii in western hemlock.*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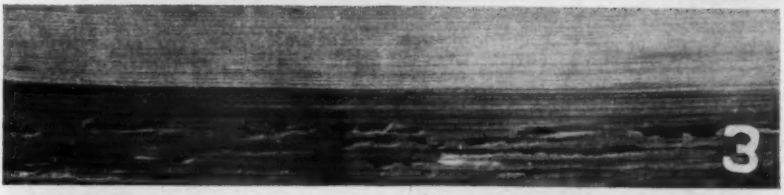
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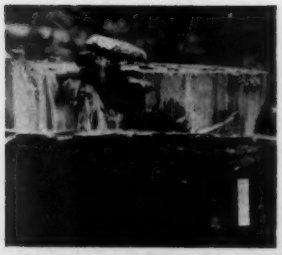
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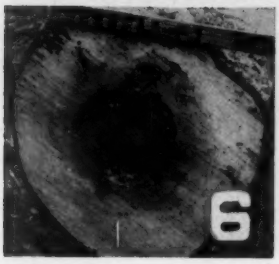
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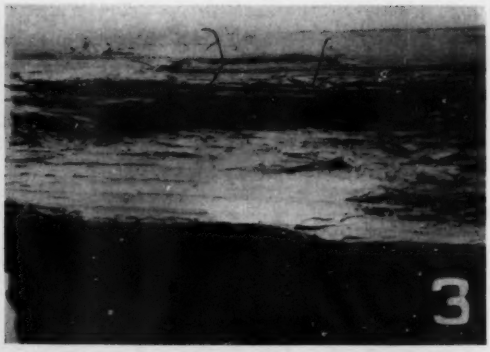
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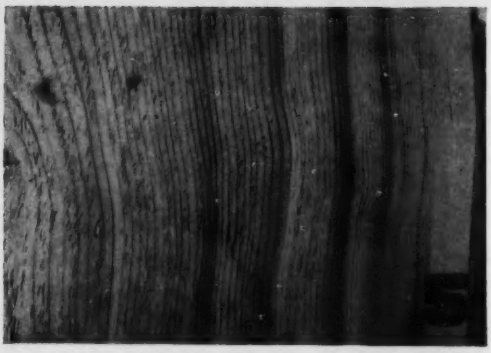
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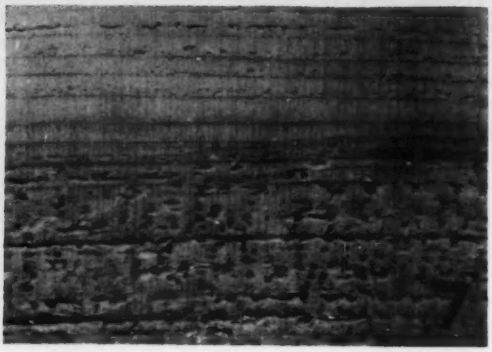

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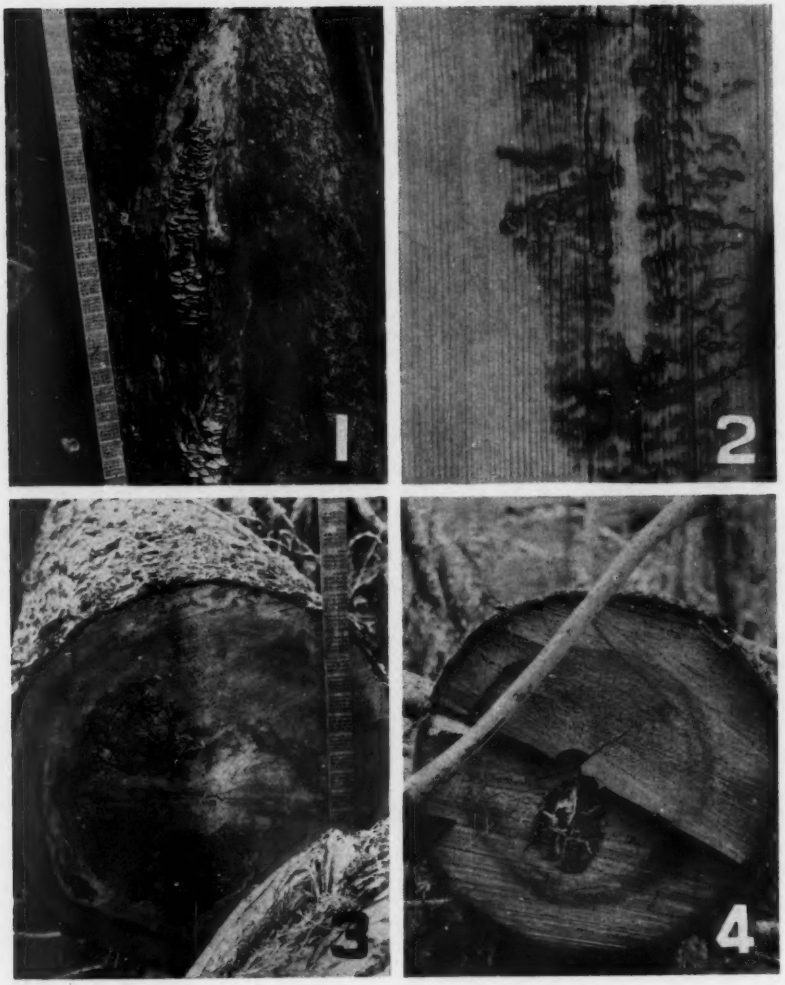
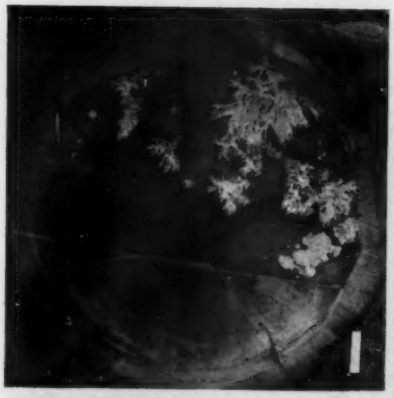
PLATE IV

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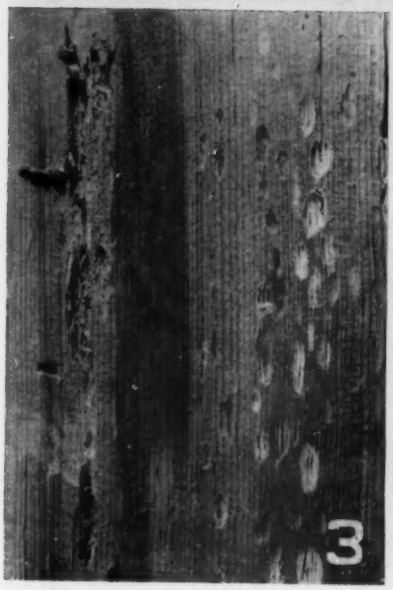
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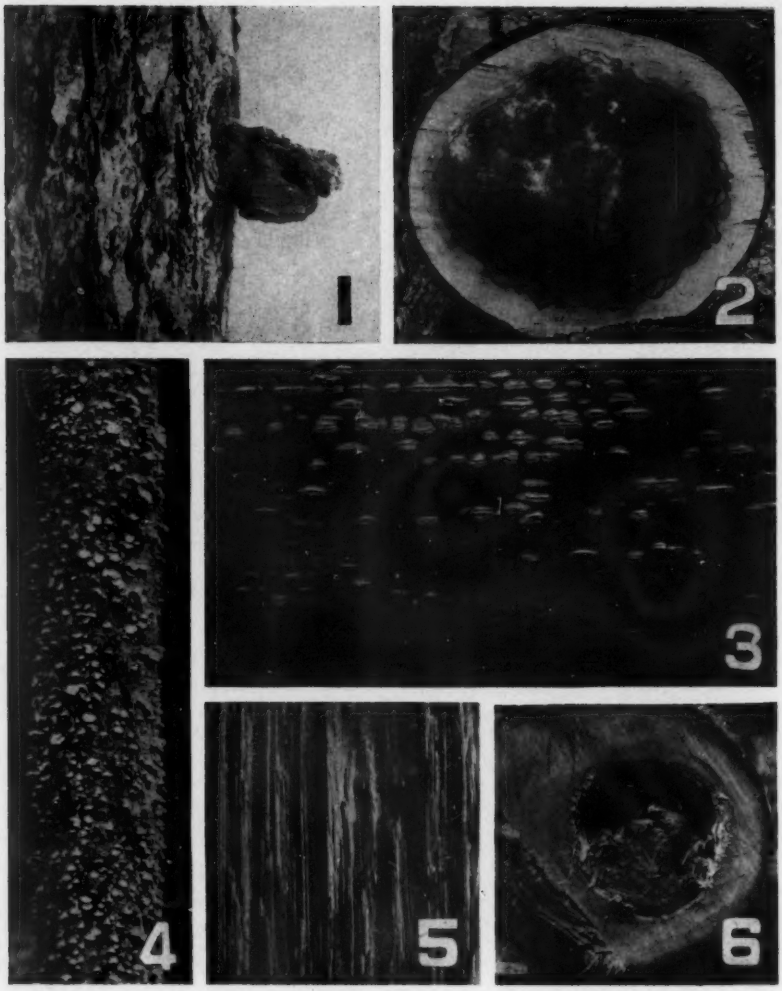
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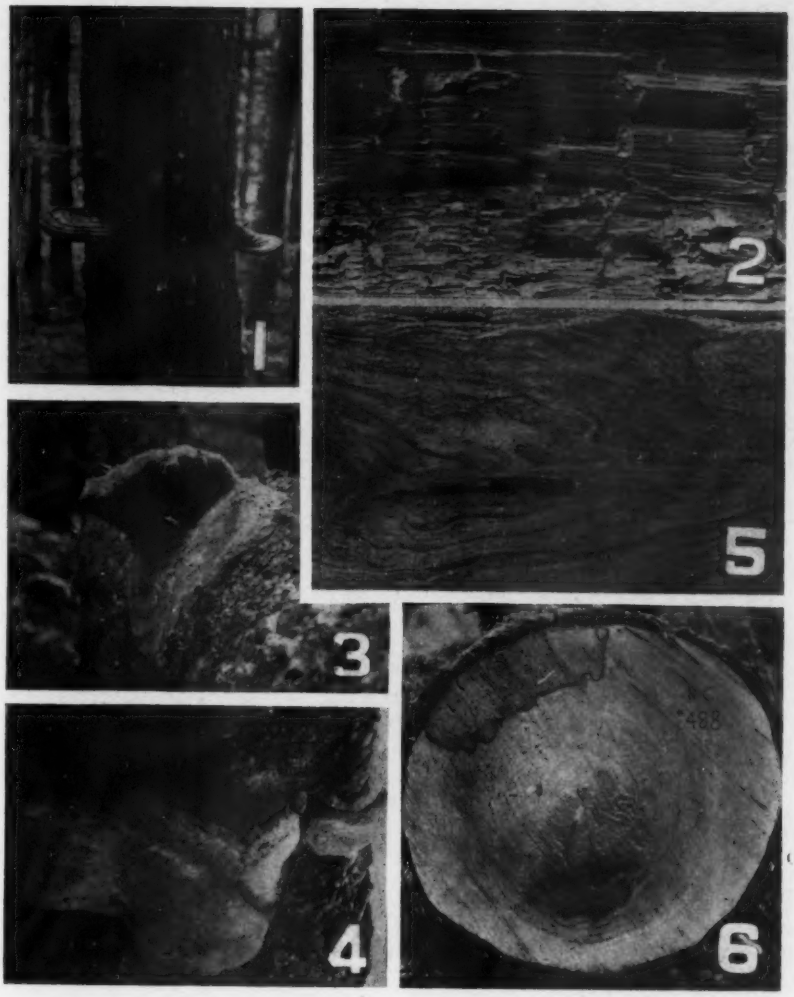
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PLATE III

Fomes pinicola and Polyporus sulphureus in living western hemlock and fir.

FIG. 1. *Fruiting bodies of Fomes pinicola on western hemlock.*
FIG. 2. *Brown cubical trunk rot caused by F. pinicola in western hemlock.*
FIG. 3. *Fruiting bodies of Polyporus sulphureus in western hemlock.*
FIG. 4. *Brown cubical decay caused by P. sulphureus with white mycelium "felts" in western hemlock.*

PLATE IV

Stereum abietinum in living western hemlock and fir.

FIG. 1. *Fruiting bodies of S. abietinum growing from a scar on fir.*
FIG. 2. *Brown cubical decay caused by S. abietinum in fir.*
FIG. 3. *Brown cubical decay caused by S. abietinum in western hemlock.*
FIG. 4. *Brown cubical pocket rot caused by S. abietinum in fir.*

PLATE V

Hydnum sp. (H. abietis) in living western hemlock and fir.

FIG. 1. *Fruiting bodies of Hydnum sp. (H. abietis) on the end of an infected log of fir.*
FIG. 2. *Fruiting bodies of Hydnum sp. (H. abietis) growing from a standing tree of western hemlock.*
FIG. 3. *Yellow pitted trunk rot caused by Hydnum sp. (H. abietis) in fir.*
FIG. 4. *Yellow pitted trunk rot caused by Hydnum sp. (H. abietis) in fir.*

PLATE VI

Fomes Pini and Polyporus abietinus in living western hemlock and fir.

FIG. 1. *Fruiting body of Fomes Pini on western hemlock.*
FIG. 2. *White pitted trunk rot caused by F. Pini in western hemlock.*
FIG. 3. *White pitted trunk rot caused by F. Pini in western hemlock.*
FIG. 4. *Fruiting bodies of Polyporus abietinus on a small dead fir.*
FIG. 5. *Stringy decay caused by P. abietinus in fir.*
FIG. 6. *Stringy trunk rot caused by P. abietinus in fir.*

PLATE VII

Fomes applanatus and Fomes Hartigii in living western hemlock and fir.

FIG. 1. *Fruiting bodies of F. applanatus on western hemlock.*
FIG. 2. *White spongy decay caused by F. applanatus in western hemlock.*
FIG. 3. *Fruiting body of F. Hartigii on a felled western hemlock.*
FIG. 4. *Fruiting body of F. Hartigii on western hemlock.*
FIG. 5. *White spongy decay caused by F. Hartigii in western hemlock.*
FIG. 6. *White spongy decay caused by F. Hartigii in sapwood and heartwood of western hemlock.*

(Plates I to VII follow.)

MUTATIONS IN POLYPLOID CEREALS

I. INTRODUCTORY OUTLINE[1]

By C. Leonard Huskins[2] and Gerhard F. Sander[3]

Abstract

Fatuoid, steriloid, and subfatuoid oats and speltoid, compactoid, and subnormal wheats arise by mutation which in most cases can clearly be shown to be a chromosome aberration. In all three segregation-types of speltoids, Series α, β, and γ, deficiency is definitely established. It ranges from the whole of one particular chromosome, designated C, down to a segment of C too small to be established microscopically, with certainty, at metaphase but still determinable genetically as a deficiency and not a single gene mutation. In the oat mutants there are similar deficiencies but the range apparently extends below the present limit of both cytological and genetic determination. At this point it naturally cannot yet definitely be differentiated from gene mutation, though no evidence favors the latter alternative. In compactoid and subnormal wheats duplication of the whole C chromosome or of its major arm is involved. Natural crossing, particularly between wild and cultivated oats, complicates the problems and is of practical agricultural significance. A distinction can, however, almost invariably be drawn between such hybrids and the mutants. The origin of the mutants and their survival and peculiar genetic behavior are all bound up with the polyploid nature of cultivated oats and wheat. This particularly affects the viability of deficient gametes. The frequency with which different types of gametes are formed is determined by meiotic behavior, which is dependent upon the particular aberrant constitution of the mutant in question; the degree of deficiency or duplication in the gametes affects their selective functioning. All these factors, together with the usual differential between ovules and pollen in the functioning of aberrant gametes, determine the diverse segregation ratios that may be obtained from phenotypically similar mutants.

Introduction

Offtype plants that resemble other closely related species occur frequently in common oats and wheat, *Avena sativa* L. and *Triticum aestivum* L. (*T. vulgare* Vill.—and herein referred to as such). The commonest of these in oats is the *fatuoid* or false wild oat, so-called because of its resemblance, in diagnostic characters of the spikelet, to the common wild oat *A. fatua* L. Less common are *steriloids* and *subfatuoids*. The former resemble the Mediterranean wild oat, *A. sterilis* L., while the latter, though not like the type of any well recognized "species", resemble specimens that have been included in *A. sterilis*, *A. fatua*, or *A. byzantina* C. Koch. These species all have 42 chromosomes, which, being three times the number found in the simplest diploid *Avena* species, makes them phylogenetically hexaploids though in most respects they segregate like diploids after hybridization. It should be added that the validity of the specific rank given to many forms of *Avena* by Linnaeus, Koch, and others is questionable, though the above-mentioned

[1] *Manuscript received in original form June 3, 1948, and, as shortened, August 3, 1949. Contribution from the Department of Botany, University of Wisconsin, Madison, Wis.*

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[2, 3] *Both formerly of the Department of Genetics, McGill University, Montreal, Que.*

ones at least are very generally referred to as species. In a modern classification, Malzew (35), however, makes all hexaploid Euavena members of the two species *A. fatua* L. *s. ampl.* and *A. sterilis* L. *s. ampl.*

In wheat the commonest offtypes are *speltoids* and *compactoids* which respectively resemble *T. spelta* L. and *T. compactum* Host. The specific rank of these forms also is of doubtful validity by modern taxonomic standards and they also are phylogenetically hexaploids with 42 chromosomes. There are in fact many parallels between the genera *Avena* and *Triticum* in their cytogenetic constitution and their apparent mode of evolution and current variation. For this reason, they are considered together in this introductory outline. In the series of detailed studies which follow in this Journal some of their commonest mutations are analyzed individually. The publication of these papers has been delayed since before the War, but they were summarized meanwhile (23) by the senior author.

A difference of great agricultural significance between the commonly cultivated forms of wheat and oats, and one which affects materially the conditions under which their offtypes are found, is that the threshed "grains" of oats characteristically consist of the caryopsis enclosed in the "flowering glumes", the lemma and palea, while the threshed wheat grain is a true grain or naked caryopsis. The two genera, however, vary alike in this, in kind though not in degree, for *T. spelta* "grains" retain their lemma and palea on threshing (as they do in more "primitive" wheat species), while they are lost in threshing *A. chinensis* (Fisch.) Metzger, a cultivated hexaploid oat and also in the "primitive" diploid *A. nuda brevis* Vavilov. It is evidently human fancy that, by selection, has determined the present characteristic difference between oats and wheat in this respect.

In wheat the awn or beard is an extension of the lemma tip while other characters involved in the mutations under consideration are revealed more sharply in the "empty" glumes than in the lemma. In oats, on the other hand, the awn arises from the mid nerve of the lemma just below its tip and the other characters are also predominantly those of the lemma. In view of this difference it is not surprising to find that mutations in wheat may affect the shape and texture, etc., of the empty glumes and the length or presence or absence of awns on the lemma either simultaneously or independently, while in oats the shape, base-type, etc. of the lemma and of the awn on it are usually affected together. Further, associated with the retention of the lemma and palea around the oat caryopsis is the fact that while both awned and awnless wheats are commonly in cultivation, almost all cultivated oats are awnless or have only very weak awns, never twisted geniculate ones such as those of *A. fatua* or *A. sterilis*. Awns on feed oats are always a nuisance, particularly to horses, while awns on wheat are a nuisance only during threshing or in the straw. It is evident, then, that both the phylogenetic development occasioned by human selection and the ontogenetic course of development favor association of awn and lemma characters in oats while freer dissociation would be expected in wheat.

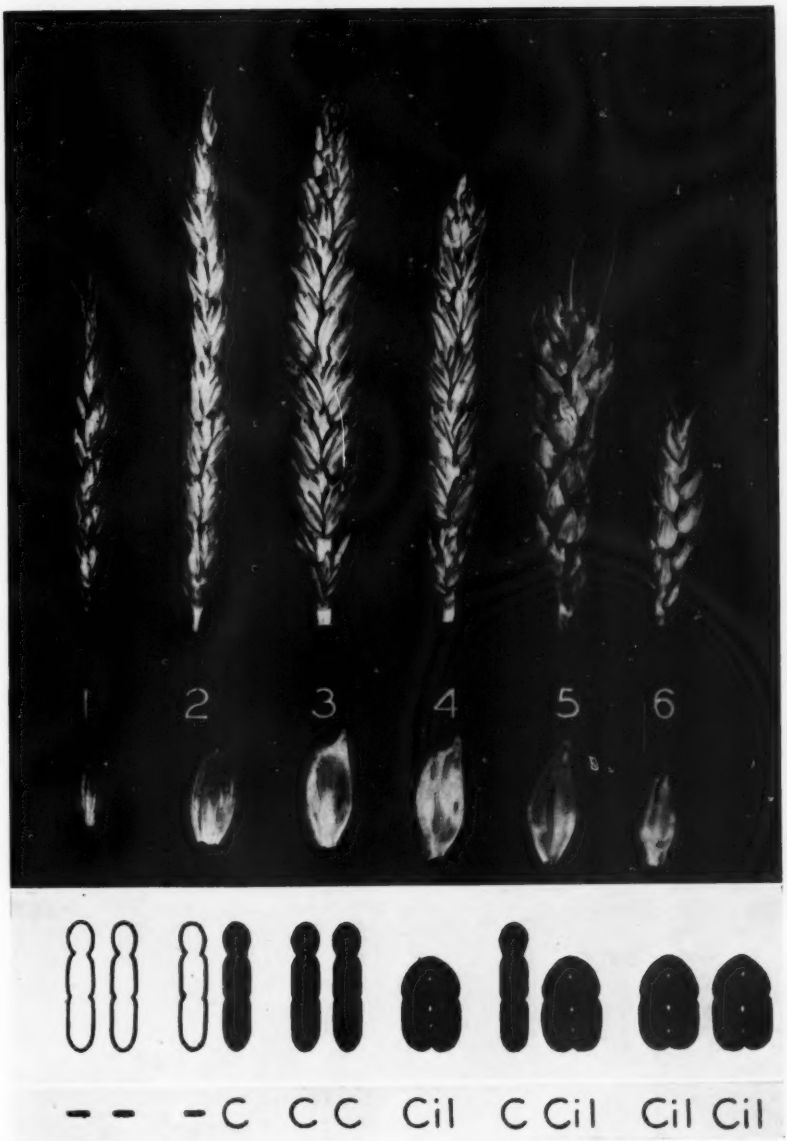
In both genera it is the floral bract characters that are chiefly used in classification of the species. In wheat the shape of the spike is a further conspicuous, though less reliable, character. Most of the other characteristics that differentiate varieties are found within all species of each, though they may differ greatly in degree of development or frequency of occurrence, especially in species that differ in chromosome number. The principal differences between the mutants and "normal" wheat and oats are shown in Plates I and II.

Historical Outline

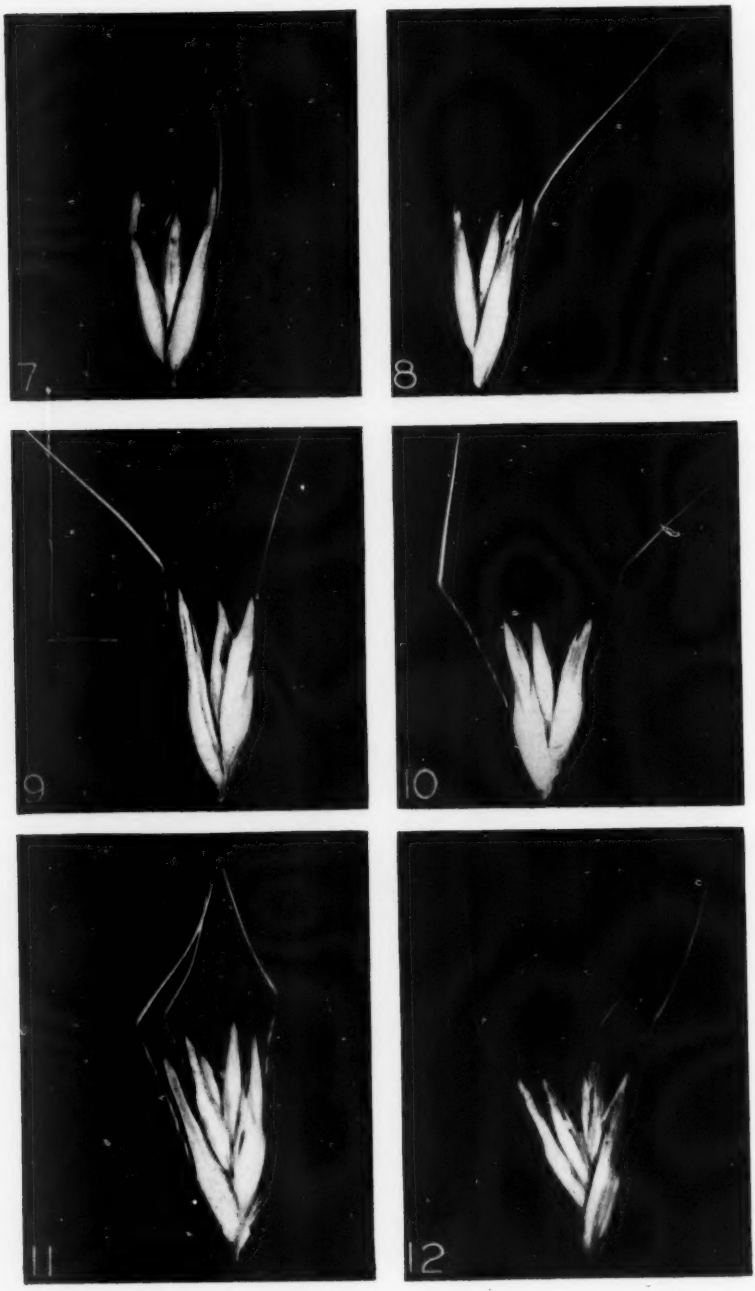
Darwin (10) quoted observations of Buckman (5) that apparently mark the beginning of the interest by evolutionists and systematists in the spontaneous variations of oats. Since 1900 both the oat and wheat variations have been the subject of numerous studies by plant breeders and geneticists. Since 1924 there have been many cytological and cytogenetic studies of them. In a recent comprehensive review of the problem (Huskins (23)), 215 contributions to it are cited. Only the most salient features and contributions will be outlined and cited herein.

Nilsson-Ehle (40 and earlier) showed that fatuoids arose in his pure lines of *A. sativa* in an area where there was no *A. fatua* and therefore no chance that they could have resulted from natural crossing with it. His genetic analysis proved them to be mutants. They differ from the variety in which they occur by only one complex of characters of the lemma and this behaves as a unit in inheritance. They are like *A. fatua* in having a strong, twisted, geniculate awn on every grain of the spikelet and a horseshoe-shaped callus ("sucker mouth") or disarticulation surface, surrounded by a tuft of hairs, at the base of each grain. Otherwise they ordinarily differ from any given variety of *A. fatua* in every respect that their parental variety differs from it, though it has been shown by Jones (25) and others that one or two other characters, especially yellow lemma color, may be genetically linked to, or physiologically associated with, the fatuoid complex in some varieties. The mutation almost always appears first in the heterozygous state. This is intermediate in most respects, there being a strong awn on only the primary grain of each spikelet and the base type being nearer *A. sativa* than *A. fatua*. The factors for the cultivated grain type being thus more or less dominant, the heterozygous fatuoid often goes unnoticed in fields of oats and the recessive true-breeding fatuoid that has segregated from it is the form most commonly found.

Tschermak (60 and earlier) has for many years argued that Nilsson-Ehle's and other similar fatuoids arise from natural crosses of *A. sativa* with *A. fatua*, but none of the evidence adduced at various times for this opinion is satisfactory. However, though in heterozygotes or hybrids the dominance of the sativa complex is slightly more complete over the mutant than it is over the fatua gene complex, the fatua and fatuoid complex of genes produce almost, if not quite, identical awns and "sucker-mouths" when homozygous. It is,



Spikes, glumes, and chromosome constitutions in terms of C and Cil chromosomes, of (1) β speltoid, (2) β het speltoid, (3) normal, (4) subnormal, (5) subcompactoid, and (6) compactoid wheats. Outline figures represent the absence of C. Cil = isomorphic chromosome consisting of a duplicated long arm of C.


7
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therefore, obvious that, from natural crosses with *A. fatua*, segregates may occur that cannot be distinguished from fatuoids that have arisen by mutation unless all characteristics of the plants are considered. Many of the offtype plants found in commercial samples of threshed oats or in fields are the result of natural crossing (Aamodt, Johnson, and Manson (1)). Under optimum environmental conditions, which include cool weather and moisture, most varieties of oats are very largely self-fertilized, but in some areas some varieties have produced as many as 6.6% of hybrid seeds as the result of natural crossing with a different variety planted in adjacent rows (Coffman and Wiebe (9)). The total amount of natural crossing, including that between similar plants which cannot be detected, must, of course, be considerably higher. The amount of crossing varies greatly between varieties and also in different countries. Data for Australia, U.S.A., Canada, U.S.S.R., Scandinavia, and Great Britain may be found in Pridham (47), Coffman and Wiebe (9), Harrington (15), Builin (6), Wexelsen (69), and Jones (26), respectively.

Nilsson-Ehle (38 and earlier) showed that speltoids also are mutants that differ from the variety of wheat in which they occur by only one complex of characters. They also almost always appear first in the heterozygous state. The speltoid "total mutation" from a tip-awned or so-called "awnless" variety (few varieties in general cultivation are truly awnless (Watkins and Ellerton, 68)) involves the appearance of awns and a thickening of the empty glume to a strongly keeled, indurated condition. The "shoulder" of the glume becomes "square" instead of "sloping"; the plant is taller and, correlatively, the spike is greatly elongated. Cultivated varieties of wheat differ considerably in these characters and the speltoid characteristics can be described more precisely only in relation to their degree of difference from the variety in which they occur. *T. vulgare* varieties range from very near the type of *T. compactum* to very near that of *T. spelta*. The speltoid mutation is a change towards the latter, the extent of which is determined by its starting point in the characteristics of the particular variety in which it occurs. Heterozygous speltoids are intermediate between the parental normal type and the true-breeding speltoid. There is much less difference in glume characters between speltoids occurring in different varieties or differing in chromosome constitution than between either heterozygous speltoids or the "normals" of different varieties.

Awnless speltoids also arise as mutants from either awned or "awnless" *T. vulgare*, and awned plants, which are otherwise like their parental type, occur in "awnless" varieties. These Nilsson-Ehle has called "part-mutations". The two part-mutations are the result of changes at different loci some 30 or so crossover units apart in one chromosome designated C herein (IX of Sears (55)). The awned speltoid type can be obtained as a crossover segregate

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Spikelets of: (7) normal, (8) het fatuoid, (9 and 10) steriloid, (11) subfatuoid, and (12) fatuoid oats. Figs. 8 and 10 are var. Banner; 7, 9, 11, and 12 var. Kanota.

from crosses between the two part mutations. Such awned speltoids arising from gene recombination continue to show crossing over between the awn and glume gene complexes while awned speltoids that arise as "total mutations" show no such crossing over. Evidently the total mutation affects both of the genes (or gene complexes) and also the interstitial region between them as Nilsson-Ehle (41) pointed out.

Genetic Ratios

It was early discovered that phenotypically similar heterozygous speltoids could differ greatly in their segregation ratios. Nilsson-Ehle (39) grouped his strains into three genetic types which he called Series A, B, and C. They will here be called α, β, and γ to avoid confusion with chromosome symbols which were introduced later in a cytogenetic study by Winge (70).

Series α comprises those speltoids whose heterozygous form after self-fertilization produces the normal (vulgare) type, heterozygous speltoids, and speltoids in ratios approaching 1 : 2 : 1. All three classes of segregates are vigorous and fertile. There are usually somewhat fewer speltoids than normals and in some strains the ratio deviates appreciably towards 1 : 1 : few. In "good" Series α strains normal and mutant gametes are evidently formed in approximately equal numbers and function almost equally well, whether on the male or female side. In strains giving ratios approaching 1 : 1 : few, the evidence indicates that the deviation from random recombination is due to mutant pollen effecting fertilization less often than normal pollen. Both types of ovule apparently function equally well.

Series β heterozygous speltoids produce three or more times as many het speltoid as normal progeny and very few speltoid offspring. These latter are characteristically dwarf and more or less sterile. Evidently Series β het speltoids produce far more mutant than normal male and female gametes but mutant pollen very rarely functions.

Series γ heterozygous speltoids, according to Nilsson-Ehle's original classification, produce slightly more normal than het speltoid offspring and very few speltoids. These rare speltoid segregates are, again, characteristically more or less dwarf and sterile but slightly less so than in Series β. For various reasons, it now seems better to include in Series γ all strains that give ratios close to 1 : 1 : few. There is no sharp dividing line between Series α and γ in their segregation ratios and this seems to apply to all of their features, including the cytological.

Until 1925 only Series α was known in fatuoids. Since then all three have been found and also a modified form of β in which the heterozygotes produce normals and het fatuoids in the usual β ratios but also numerous, instead of few, fatuoid offspring, though these are dwarf and sterile as in typical Series β. Evidently the mutant pollen produced by heterozygous fatuoids of modified β functions as well or better than their normal pollen, but the homozygous mutant, besides being dwarfed, produces abortive pollen and is almost completely sterile.

In wheat there is little difference in the frequency with which the three Series have been found. All speltoids are discovered as plants, not as threshed grains, since these lack the diagnostic glumes, and they are usually noticed as heterozygotes as soon as they occur in plantbreeders' plots since they are taller than their parental stock. The frequency of discovery of the mutants is therefore probably a fair indication of the frequency of occurrence of the mutation. This does not hold for fatuoids as it is usually the homozygous segregates that are discovered either in the field or as threshed grains. These are dwarf and sterile in Series β and γ. Heterozygous fatuoids are usually similar in height to their parental stock and they are not very easily noticed in the field. In threshed stocks the grains of the spikelets are separated and the awns broken off. The secondary and tertiary grains of het fatuoid spikelets cannot be distinguished from the normal after they are separated from the primary and the latter are not always strikingly different after the awn is broken off. It is therefore not surprising that most fatuoid strains belong to Series α whether they are first found as plants or seeds. Series β and γ strains have almost all originated from definite searches for heterozygous fatuoid seeds or plants, though the Series to which they belong cannot be determined from the heterozygous phenotype.

Compactoids usually arise in the heterozygous, intermediate state here designated subcompactoid, and their commonest source of origin is as infrequent and irregular but characteristic segregates from Series β, or more rarely Series γ, het speltoids. They can arise, however, directly from pure lines of normal *T. vulgare*. Subcompactoids that are phenotypically similar may belong to types that segregate very differently. The simplest type, after the customary self-fertilization, gives only normal, subcompactoid and compactoid offspring. Another type gives in addition a small proportion of het speltoids. A third, the commonest, gives normals, subnormals, het speltoids, subcompactoids, and compactoids. The ratios are very irregular and attempts to determine the cause by genetic analyses alone met, not surprisingly, with very little success. They are now known to have complex chromosome constitutions (Håkansson (12, 14); Smith (56, 57); Huskins (22, 23)). Correlatively, their genetic analysis is complicated by much sterility and outcrossing of semisterile plants. In addition there is, as with speltoids only more so, the complication that the types can be classified only in relation to the norm of the variety from which they originated. The subcompactoid arising in a variety of *T. vulgare* with moderately dense spikes and narrow glumes may, for instance, closely resemble in glume and spike characters the compactoid mutant of a long-headed, wide and square-glumed variety while at the same time being very similar to the normal phenotype of a dense-headed, narrow-glumed variety of *T. vulgare* that approaches the type of *T. compactum*.

All of these wheat and oat mutations can, of course, occur either in pure lines or in hybrid stocks. If in the latter, their analysis may be very complex. They can also occur either germinally or somatically. If the latter, the result

is a mosaic plant or chimera. The chimeras found most commonly in wheat are sectorials having one side of the head normal and the other het speltoid. A somatic mutation that produces entire heterozygous mutant culms or tillers, leaving other culms normal, can be discovered only in studies that involve the examination of individual plants and since twin seedlings are not uncommon in wheat (Muntzing (37)) and double oat grains are very common, it is difficult to establish definitely that plants with whole heads of different types are the product of one embryonic zygote. Many chimeras of diverse types have been found by Åkerman (3) and others, including the writers. As the diagnostic characters of all the mutants are maternal tissue, the phenotype of the parent plant does not necessarily give any indication of the genetic constitution of the seeds within the mutant or normal floral bracts. The glumes in wheat are produced by the dermatogen and the seeds by subepidermal layers according to Rösler (48). A periclinal chimera that looked like an ordinary het speltoid but had only the outer cell layer mutated would therefore produce only normal offspring. The ratio of mutant and normal types produced by het speltoid or het fatuoid spikelets or grains of a mosaic spike or panicle rarely gives any indication of the ratio their heterozygous offspring will produce, since the mutation may have affected only the epidermal layer in some of the grains and both the epidermal and subepidermal in others.

Cytological Interpretations

Nilsson-Ehle classed both fatuoids and speltoids as "loss-mutations" and more specifically, as "complex-mutations" resulting, he thought, from the loss of a "complex" of genes that is necessary for the development of the normal phenotype of cultivated oats and wheat. As early as 1920 he speculated that they might be due to the loss of chromosome segments as, according to the then recent genetic analyses of Bridges, were the "deficiency mutations" of *Drosophila melanogaster*. In this he was remarkably prescient but, speaking generally, though he, Lindhard (31 and earlier), Åkerman (2), and many others proved the mutational origin of the various offtypes and by genetic analyses elucidated many of the peculiarities of the different Series, it was not until correlated cytological and genetic studies were made that the most puzzling features could be clarified and the problem as a whole be understood. The first cytological study was made by Winge in 1924 on various strains obtained from Lindhard and one from Åkerman.

In 1917 Winge had pointed out the very significant role played in plant evolution by chromosome doubling following hybridization, which is now known either as allopolyploidy or amphidiploidy. At that time the chromosome number of wheat had been given as $2n = 16$ by nine different cytologists. Little was then known of polyploid genetics and, in any case, on the basis of these counts wheat would have been considered a diploid species. Varying numbers up to 44 had been counted in oats. In 1918, it was, however, established independently by Sakamura (49) and Sax (53) that in *Triticum* there are three groups of species with 14, 28, and 42 chromosomes. In 1919

Kihara (29) proved that a parallel situation exists in *Avena*. The cultivated species so far mentioned herein have 42 chromosomes and since these regularly form 21 bivalents at meiosis the cytological presumption is that they are amphidiploids, not autopolyploids that have resulted from chromosome doubling in one species. The results of many genetic analyses of hybrids between the different groups support this (see Sears (54)) and recently Thompson (Thompson, Britten, and Harding (59)) has synthesized a *T. vulgare* type by doubling the chromosome number artificially in a hybrid of *T. turgidum* ($n = 14$) $\times$ *Aegilops speltoides* ($n = 7$). Less is known of *Avena* phylogeny but it has, for instance, been shown by Jones (27) that the awn and lemma characters which are associated in *A. fatua* and fatuoids occur as separately inherited characters in certain diploid and tetraploid oat species. This could be taken to indicate that the geniculate awn and horseshoe base of *A. fatua* grains are due to factors that came into it from two simpler species. We shall see that the mutation data indicate that these same factors are probably present in normal *A. sativa* where their effect is inhibited by a single "cultivated factor" or a closely linked group of factors. When this is lost the fatuoid characters appear.

In his preliminary study of Åkerman's Series α speltoid strain, Winge (70) found only the normal formation of 21 bivalents in pollen mother cell meiosis. But later in Lindhard's α speltoids he occasionally found a quadrivalent and 19 bivalents and in heterozygous speltoid sibs there were sometimes 19 bivalents, one trivalent, and an unpaired chromosome. These were taken to indicate the presence of four identical chromosomes in the speltoids and of three identical and one odd chromosome in the heterozygous speltoids. On this basis Winge constructed an ingenious hypothesis. That it is now known to be incorrect in detail does not detract unduly from its value, for it has led the way to a fuller understanding not only of the speltoid and fatuoid problems but of polyploid genetics in general.

Winge pointed out that the amphidiploid set of *T. vulgare* may be assumed to comprise three similar, but not identical, paired sets, or genomes, of seven chromosomes each, which have been derived from three ancestral diploid species. If the three origins are designated A, B, and C, and the chromosomes of a basic set or genome of the genus *Triticum* are numbered 1 to 7, then the 21 pairs of *T. vulgare* may be represented as

$$\frac{1A}{1A}\ \frac{1B}{1B}\ \frac{1C}{1C};\ \frac{2A}{2A}\ \frac{2B}{2B}\ \frac{2C}{2C};\ \ldots\ \frac{7A}{7A}\ \frac{7B}{7B}\ \frac{7C}{7C}.$$

Only one multivalent chromosome association was seen in the mutants and the genetic analyses indicate that the change producing the mutation has involved only a single factor or one group of linked factors. Therefore only one of the triplicated pairs of chromosomes need be considered; Winge simply dropped the numeral and called it $\frac{A}{A}\ \frac{B}{B}\ \frac{C}{C}$. Since pure lines and simple Mendelian hybrid ratios occur in *Triticum vulgare*, chromosome A must

normally pair with A, B with B, and C with C. Winge's primary postulate for the origin of speltoid mutants was, however, that B and C may occasionally pair (or the two B's and two C's form a quadrivalent) since, though not identical, they must be phylogenetically similar. At that time meiotic pairing was generally looked on as a measure of general "affinity" between chromosomes that were themselves units. We now emphasize identity of chromosome segments as a primary factor in synapsis during the meiotic prophase and association of whole chromosomes at metaphase as a secondary phenomenon, usually as a result of chiasmata (exchanges of partners) being formed between identical segments when crossing over occurs during prophase. We should now expect the phylogenetically similar B and C chromosomes to have many identical segments but the arrangement of these to be different and some segments to be present in the one that are lacking in the other. To distinguish chromosomes that for phylogenetic reasons have certain segments in common, and are therefore capable of pairing occasionally, from chromosomes that are regular pairing partners or homologues, we may call the former "homoeologues" (Huskins (20)) (homos = the same; homoios = similar).

If pairing takes place between B and C, then, Winge pointed out, gametes ABB and ACC may be formed. The gamete ABB united with a normal gamete ABC forms the zygote $\frac{A}{A}\ \frac{B}{B}\ \frac{B}{C}$, which Winge gave as the formula of a Series α heterozygous speltoid. This was taken to account for its occasional formation of a trivalent, BBB, and a univalent, C. It would, he assumed, segregate normals, $\frac{A}{A}\ \frac{B}{B}\ \frac{C}{C}$, heterozygous speltoids, $\frac{A}{A}\ \frac{B}{B}\ \frac{B}{C}$, and speltoids, $\frac{A}{A}\ \frac{B}{B}\ \frac{B}{B}$, in irregular ratios but approximating 1 : 2 : 1. The four B chromosomes of a speltoid segregate would, of course, form its quadrivalent. The irregularities of pairing in these abnormal chromosome mutants, or *aberranten* as he called them, would be expected to produce other abnormal types of gametes such as ABo and A (BC) C, the former entirely lacking a C chromosome and the latter having a composite (BC) formed by interchange of parts between B and C during the time they were paired.

In Lindhard's "squarehead heterozygote" or "subnormal" type and also in a "perennis" type, which formed a big rosette of leaves and few heads, Winge found only 41 chromosomes. In a subcompactoid he could sometimes count only 41 chromosomes, but in other cells there were 42. He decided that one must sometimes get lost and degenerate in the cytoplasm.

From the point of view of developmental genetics Winge followed Nilsson-Ehle's lead in assuming that the "normal" phenotype of *T. vulgare* is determined by a balance between factors some of which push development towards the spelta and others towards the compactum type. The B chromosomes predominantly carry the former and C the latter factors, Winge assumed.

He expected therefore to find some heterozygous speltoids resulting from the loss of a C chromosome, i.e. of the constitution $\frac{A}{A}\ \frac{B}{B}\ \frac{o}{C}$. Oddly enough he found none, though this is now known to be the formula of β heterozygous speltoids, to which Series most of Lindhard's stocks belonged. Ironically, it is the only formula of the 14 which Winge gave that now seems to be thoroughly substantiated. In most of the other mutant types, as will be shown in later papers of this series, structural changes have taken place in the C chromosome. In others there are changes in number or balance but apparently not of the precise type that Winge assumed.

Goulden (11) and Huskins (17) had independently begun cytogenetic analyses of fatuoids very shortly after Winge began his cytological study of speltoids. Goulden found that in dwarf fatuoids which would now be classified as modified Series β, the pollen mother cell meiosis was grossly abnormal. He suggested that "at least part of a chromosome which also carries the cultivated factor has somehow been lost". Huskins (18) adopted Winge's interpretation for Series α fatuoids. He found β and γ strains, hitherto unknown in oats, and determined that the former lack a C chromosome; he thought erroneously — see later — that the latter had an excess of B chromosomes. In a subsequent study of speltoids (19) a similar interpretation for the three Series was given.

Nilsson-Ehle (41) pointed out weaknesses in Winge's hypothesis that were revealed by his own genetic studies of "part-mutations", i.e. beardless speltoids and bearded normals.

Håkansson (12, 13), Muntzing (36), Phipps and Gurney (46), and Uchikawa (64) have confirmed Huskins' observation that Series β het speltoids are monosomics – lacking one C – and that the dwarf speltoids segregating from them are nullisomics – lacking both C chromosomes. Nishiyama (42) has confirmed this for modified β fatuoids. Vasiliev (65) reported the same constitution for a γ speltoid strain studied genetically by Philiptschenko (45) but further analysis of it by S. G. Smith and the present authors have shown that the monosomic and nullisomic plants examined by Vasiliev must have been β segregates. These are produced in irregular numbers by all γ strains and, as shown in the second paper of this series, they occur more frequently than usual in this strain. Final confirmation that loss of the C chromosome produces speltoids has come in the recent analysis by Sears (55) of 17 wheat monosomics and their corresponding nullisomic types. The C chromosome is his number IX.

In one γ het speltoid examined in his original study (19), Huskins observed a heteromorphic bivalent but thought it was not involved in the determination of the speltoid characteristics. In many pollen mother cells of both het speltoids and speltoids he could find only the normal chromosome number but in others there appeared to be 43 and 44, respectively. These higher numbers were taken to be the correct ones (as with the techniques then in use counts

of the entire complement were difficult) and by the same extension of Winge's hypothesis as used for γ fatuoids (18), the constitutions of het speltoids and speltoids of Series γ were given as $\frac{A}{A}\ \frac{B}{B}\ \frac{\ }{B}\ \frac{C}{C}$ and $\frac{A}{A}\ \frac{B}{B}\ \frac{B}{B}\ \frac{C}{C}$, respectively. Muntzing (36), Håkansson (12), and Bynov (7) also recorded 43 and 44 chromosomes in other γ strains. Very soon, however, Huskins (21, 22) discovered that it was the heteromorphic bivalent that was significant and that the counts of 43 and 44 chromosomes were in some cases erroneous and that in others they resulted from the presence of extra chromosomes having little or no effect on the speltoid characters. Shortly afterwards Nishiyama (43, 44) and Uchikawa (61, 62) independently found that γ fatuoids and speltoids characteristically have a heteromorphic bivalent but the normal number of chromosomes. It is now clear that γ speltoids and fatuoids arise through loss of part of the C chromosome. This is most readily detectable in heterozygotes at the first meiotic metaphase when the deficient C is paired with a normal C forming a heteromorphic bivalent. The deficiency varies greatly in size in different strains, sometimes with correlative genetic results in the segregation.

Extensive cytological studies of α fatuoids and speltoids were made by Nishiyama (42) and Uchikawa (64). They found multivalent associations but concluded that these bore no relation to the fatuoid or speltoid characters. Though they agreed with Huskins that Series β and γ arise through whole chromosome loss and segmental deficiency respectively, they decided that Series α strains originate by gene mutation. Jones (24) also favored this interpretation, but later (27) favored the assumption of a small deficiency in chromosome C as postulated by Muntzing (36) and by Huskins (22) after his discovery of variable-sized deficiencies in Series γ. On this later interpretation the speltoid or fatuoid phenotype of any Series arises through the loss of "cultivated factors" or "wild-type inhibitors" while the extent of the deficiency determines their diverse segregation (whole chromosome deficiency gives β, large segment γ, and minute segment α). Detailed cytological and genetic evidence for this will be presented in subsequent papers of this series.

Since the phenotype of *T. vulgare* is the result of a balance between factors with opposing tendencies there is nothing to be said, on *a priori* grounds, against the hypothesis that speltoids or fatuoids may arise through duplication or substitution as originally postulated for α and γ, instead of by deficiencies. Sears (55) has found that chromosomes II and XX carry some of the factors postulated by Winge to be on the B chromosome. Continuation of his line of attack on the problem of the contribution of individual chromosomes to the phenotype of *T. vulgare* will give a definite answer. There remains for consideration here the question of the nature and significance of the multivalents found by Winge (70) and Huskins (18, 19) in α speltoids and fatuoids.

Some of the observations of trivalents and quadrivalents made by the senior author were erroneous, due either to inexperience or to the use of the paraffin-section method now superseded by the squash technique, which is

greatly superior for this material and purpose. Other multivalents, however, were accurately described and their occurrence has been abundantly confirmed by later observations of many workers on diverse α strains. The problem of interpreting the significance of these in relation to the mutations hinges on their relative frequency. Nishiyama (42) and Uchikawa (64) concluded, as mentioned, that in their α strains multivalent formation and other irregularities of meiosis were no more frequent in the mutants than in their normal sibs. But comparisons, to be conclusive, must be between a normal variety and het speltoids that have newly arisen from it, for if, as postulated by Winge, the constitution of an α het speltoid were originally $\frac{A}{A}\ \frac{B}{B}\ \frac{B}{C}$ and pairing therein commonly took place between B and C they would exchange segments and the C chromosomes of an extracted normal would no longer be as restricted in their pairing potentials as they originally were. Nor would the four B chromosomes of the postulated $\frac{A}{A}\ \frac{B}{B}\ \frac{B}{B}$ speltoid segregates be segmentally identical. These are, however, theoretical considerations which are unnecessary for the understanding of the α genetic ratios (we cannot *in any particular case* expect to know definitely the *mode of origin* of the deficiency which determines its cytogenetic characteristics). Further, wheat and oat varieties vary greatly in their regularity of chromosome pairing and the total frequency of irregularities found by either Nishiyama or Uchikawa in any of their material is lower than that observed in some pure varieties and many intervarietal and interspecific oat or wheat hybrids—see Hollingshead (16), Thompson (58), Vasiliev and Kamenik (66), Camara (8), Katterman (28), Love (34), and others. Sapehin (52) reported that one "high yielding pure line of spring wheat has 20-30 per cent and up to 50-60 per cent in some years of pollen mother-cells with disordered arrangement of chromosomes". On the other hand, bivalent formation, and meiosis in general, is usually regular in some varieties and strains of wheat and oats.

The "speltoid" strain in which Huskins (19) found the most abundant and clear-cut cases of multivalent formation was at that time suspected of being a natural hybrid between *T. vulgare* and *T. spelta* or some derivative of this cross. This has since been confirmed by Dr. Å. Åkerman in whose cultures the crossing occurred. Many multivalents and irregularities have been found in other natural hybrids occurring in our fatuoid or speltoid strains. It is interesting therefore that a recent re-analysis of Lindhard's and Winge's papers has led the authors to suspect that the strain most extensively examined by Winge may also have been hybrid. The evidence is as follows:

Lindhard's het speltoids were originally of Series β, giving about one normal to eight het speltoids and for the first four generations no speltoid offspring. In D_4 a bearded het speltoid arose. It and a beardless sib gave respectively 6 : 44 : 51 and 9 : 63 : 14 normal, beardless het speltoid and bearded speltoid progeny in D_5. The latter were all vigorous plants, unlike the sterile dwarf speltoids which Lindhard occasionally got in later generations from typical

Series β lines. The beardless het speltoid was taller than any of its sibs. Sixteen of its beardless het speltoid progeny gave a typical Series β ratio, 29 N : 252 H, but one of the latter was again bearded, while 16 bearded speltoid sibs gave 105 bearded speltoid and four beardless speltoids in D_6. In D_7 two beardless het speltoids gave Series β ratios, but their offspring included one bearded het, one bearded speltoid, and two beardless speltoids while three others gave ratios like those of D_5, totalling 29 normals, 161 het speltoids, and 49 bearded speltoids. In all generations there was great variability in height and vigor, especially of the het speltoids. In crosses between speltoids of these lines and normal or compactoid types, the het speltoid F_1 plants all gave Series β ratios in F_2 if the speltoid was the pollen parent. The reciprocal cross gave some Series β het speltoids and some that segregated like the D_4 ancestors to give a number of vigorous speltoid offspring.

These data indicate either natural crossing or the presence of extra chromosomes. Certainly these speltoids, which were the ancestors of the plants from which Lindhard sent cytological material to Winge, were not typical α either in origin or genetic segregation. It appears significant that Winge found no multivalents in an α strain that he obtained from Åkerman and that Lindhard recorded that his "speltoids" had hard and tight glumes like those of typical *T. spelta*. In a personal communication to the senior author in 1927 he went further and expressed the opinion that there is no difference between speltoid and *T. spelta*. This conclusion is inacceptable (see Watkins (67)) but it would come naturally to Lindhard if the vigorous plants that he considered to be speltoids segregating from self-fertilized β het speltoids were really the offspring of cross-pollinations by either *T. spelta* or derivatives from it.

Compactoid wheat mutants result from duplication of the factors that are deficient in α, β, and γ speltoids. Håkansson (12, 14) found two chromosome types. In one the subcompactoid is a trisomic having three C chromosomes and the compactoid segregates from it are tetrasomic for C. In the other it is only the long arm of the C chromosome that is duplicated; this occurs through the formation of an isochromosome, i.e., one having two similar arms. These and several other types with diverse replications of part or all of the C chromosome were found coincidently in our material (Huskins (22)) and later by Katterman (28) and Uchikawa (63).

Love (33) and Sander (51) have shown that the steriloid and subfatuoid mutants arise through alterations in the C chromosome of *A. sativa*. Provisionally they can be considered part mutations of the fatuoid complex. The three types behave like a multiple allelic series (Sander (50), Jones (27)) but it is doubtful that they are due to modifications of a single locus.

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studies were continued at King's College, London, and the John Innes Institution. Since 1930 they have been continued as a sideline interest, carried on jointly with research students — especially J. M. Armstrong, E. A. Kerr, R. M. Love, S. G. Smith, and H. G. F. Sander — see (4, 33, 56, 57, 51). From 1931-35 plants were grown at Macdonald College, Que.; from 1937-44 at the Montreal Botanical Garden. The Department of Agronomy, University of Wisconsin, is currently providing facilities for maintenance of stocks and studies of new issues. For these facilities and help and for grants from the National Research Council of Canada, thanks are here expressed.

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MUTATIONS IN POLYPLOID CEREALS

II. THE CYTOGENETICS OF SPELTOID WHEATS[1]

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Abstract

The bearded speltoids that are found in beardless varieties of *Triticum vulgare* are usually segregates from heterozygous speltoids that have arisen through mutational loss of two genes, or gene complexes, that normally determine the glume and, to a lesser extent, the lemma and rachis characteristics that predominantly differentiate *T. vulgare* from *T. spelta*, which is possibly one of its ancestors. These "vulgare" genes are epistatic to genes that in their absence determine indurated, sharply and heavily keeled glumes and bearded lemmas closely resembling those of *T. spelta*. The two "vulgare genes" are both on one chromosome ("C" or "IX"); the hypostatic "spelta" or speltoid genes are probably multiple and have not yet been definitely located.

The "vulgare" genes or gene complexes may be lost independently, giving (after segregation) "part-mutations", i.e. beardless speltoids or bearded normals, or they may be lost together, giving the "total mutation" i.e. bearded speltoid. The mutations that produce the "part-mutants" are necessarily segmental changes, but the total mutants may arise through either segmental or whole chromosome loss.

Loss of an entire C chromosome gives a β het speltoid which, on selfing, gives normal, het speltoid, and bearded speltoid offspring in a ratio varying about the mode 1 : 5 : few. These β bearded speltoids are dwarf, sterile nullisomics having only 20 pairs of chromosomes, $20_{II} - -$, in place of the 21_{II} normal for *T. vulgare*. The characteristic β Series ratio and its variations are mainly determined by the frequency with which the unpaired C of a β het speltoid is left out of the gynospore nuclei during meiosis and by the lower functioning of 20-chromosome pollen. Zygotic elimination plays a minor role.

Deletion of an appreciable interstitial segment, or the whole, of the long arm of C produces γ het speltoids which on selfing give normals, het speltoids, and speltoids in ratios near 1 : 1 : few. If the segment deleted involves both gene complexes the speltoid segregates are bearded and more or less dwarfed and partially sterile. If it does not involve the beard "inhibitor" they are beardless. The γ ratio is determined largely by the lower functioning of pollen carrying the deletion.

Deletion of a segment of C too small to be established definitely at metaphase but genetically determinable as of not less than about 30 cross-over units in length gives α het speltoids that segregate normals, het speltoids, and bearded speltoids in ratios approaching 1 : 2 : 1 and all segregates are of normal size and fertile. Evidently this deletion does not materially affect pollen functioning.

Hybridization and polyploidy have been involved in the evolution of *T. vulgare* and it is the latter which permits the functioning of gametes bearing deficient chromosome complements and the survival of aberrant types that would be unlikely to exist in a diploid species. The hybrid ancestry accounts for quantitative chromosome changes being able to produce forms that closely resemble ancestral or related species, and also for the fact that crosses between varieties of *T. vulgare* or of closely related species can produce forms that may be phenotypically indistinguishable (or nearly so) from the mutants.

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I. Introduction

The speltoid mutants of *Triticum aestivum* L. (*T. vulgare* Vill.) here analyzed fall into three main segregation types, Series α, β, and γ. The basis of classification is a slight modification of the original A, B, C of Nilsson-Ehle (24). The symbolism is changed not because of the modification but to avoid confusion with chromosome and gene symbols. Heterozygous speltoids of Series α on self-fertilization give normal, het speltoid, and speltoid progeny in ratios approaching 1 : 2 : 1. The ratios deviate from 1 : 2 : 1 in having a greater or lesser deficiency in the het speltoid and speltoid classes. Series β het speltoids produce mainly normal and het speltoid progeny. In different strains and under varying conditions, the ratio of these varies from 1 : 3 to 1 : 8 or more. Speltoid progeny are either rarely produced or rarely survive to maturity, and, if they do, are characteristically dwarf and sterile. Series C het speltoids on Nilsson-Ehle's definition produce slightly more normal than het speltoid progeny while their speltoid progeny are rare and nearly always more or less dwarf and sterile, though they are slightly more numerous, taller, and more fertile than in Series β. None of the strains received as Series C from various sources, including Nilsson-Ehle himself (see Table I), have given more normals than het speltoids under our conditions or those of Uchikawa (36) to whom seeds of Nilsson-Ehle's Series C Strain 26-1295 were sent. We class as Series γ all strains that give het speltoids and normals in ratios closely approaching 1 : 1 while producing few speltoid progeny and these more or less dwarf and sterile. The difficulty of determining the frequency with which the three Series arise directly from normal wheat was discussed in preceding accounts of the history and general features of the problem of recurring mutations in polyploid wheat and oats (11 and 12). In Nilsson-Ehle's cultures prior to 1921, the three Series had each arisen five times as het speltoid mutations from standard varieties or from lines selected from crosses between standard varieties of wheat. In subsequent generations, however, the ratio-type is not necessarily constant. The most frequent change is from γ to β. This is now known to be due to occasional failure of pairing between the segmentally deficient C chromosome, which is characteristic of Series γ, and its normal homologue. This results in the formation of some ovules entirely lacking a C chromosome. One of these fertilized by a normal pollen grain produces a Series β het speltoid. A change from Series β to γ has several times been reported (24, 16, 17), but it has not occurred in our cultures. One way in which it could occur would be by the loss of the longer arm of the univalent C chromosome of a Series β het speltoid through misdivision of its kinomere and combination of the new "Cts" chromosome with a normal C. Many of the changes in ratio-type may, however, be due to cross fertilization which occurs with rather high frequency on plants which form defective pollen.

The complete speltoid or "total-mutation" dealt with herein involves a complex of glume and awn characters, the speltoids being heavily bearded

and the normals "beardless" or, more strictly, "awn-tipped". Two or more linked factors are involved and no crossing over occurs between them in self-fertilized het speltoids or crosses of their speltoid segregates with normals or other types. Hence Nilsson-Ehle in 1921 termed the process "complex mutation", by which he was referring not to complexity of the ratios, etc. but to the fact that the mutation involves a complex of factors. He considered the possibility (now established) that it might be similar to the deficiency mutations then recently discovered in *Drosophila*.

Cytologically, Series β is the simplest type, since its het speltoids result from the loss of one whole C chromosome. Series γ het speltoids result from a gross deficiency in one member of the pair of C chromosomes, giving a clearly heteromorphic bivalent at first meiotic metaphase. Series α most probably results from a small deficiency but this is difficult to establish cytologically in most strains and the analyses of it are therefore the least satisfactory. The observations on the three series will therefore for purpose of clarity be presented in the order β, γ, α.

II. Material and Methods

Most of the strains were obtained from Prof. H. Nilsson-Ehle or Dr. Å. Åkerman, Svalöf, Sweden; one γ Series from Prof. Jur. Philiptschenko, Leningrad, and another from Dr. S. J. Wellensiek, Wageningen, Holland; see Table I.

Unless otherwise indicated, all plants described and progenies recorded have been grown in the field in rows six inches apart, with 12 seeds four inches apart in each row. "Germination" records are made soon after the seedlings appear above ground.

Until 1930 most cytological preparations were fixed by Kihara's (Carnoy and Flemming) method, paraffin-embedded, sectioned, and stained with Newton's iodine–gentian violet. Since 1930 the permanent acetocarmine smear method of McClintock has been used. S. G. Smith found in 1936 that it is possible to obtain excellent acetocarmine smears, greatly superior to sections, from the old paraffin-embedded material. This has been valuable for checking earlier observations. The material is run through three changes of equal parts xylol and *N*-butyl alcohol. After two changes in normal butyl alcohol (and bleaching in equal parts hydrogen peroxide and butyl alcohol if the fixative contains osmic acid), it is run down to 70% ethyl alcohol and smear preparations then made.

The following abbreviations are used for the phenotypes: Sp = speltoid, H or Het Sp = heterozygous speltoid; N or Normal = normal appearance for the parental variety regardless of the differences between varieties; SN = subnormal or "square-head heterozygote" of other authors; SC = sub-compactoid; Comp. = compactoid.

TABLE I

ORIGIN AND INTERRELATIONSHIPS OF THE SPELTOID AND COMPACTOID WHEAT STRAINS

Series and strain	Donor and year received	Donor's No. and new number	Information from donor: Originated As	Information from donor: Originated From	Information from donor: Strain or parent stock	References and relation to other strains
β 1	Åkerman 1926	1924-440 26-53/55	Het B	Het A	Line 0715 ex Börsum	Gave SC Strain 3 (35)
β 2	Åkerman 1926	1926-497 26-72	Het B in 1926	F_8	Extrakolben × Br. Schlanstedt	Sib of α 1
β 3	Åkerman 1926	1924-921 26-67/71	Het/N chimera in 1919	Het B	Landweizen	Åkerman (2) p. 326
β 4*	Nilsson-Ehle 1927	26-1292 26-92/94	No information; apparently from same variety as γ 4			
γ 1	Åkerman 1926	1924-444 26-62/66	Het A	N	0201 × Börsum	Gave SC Strain 2 (35) Hakansson (6)
γ 2	Philiptschenko 1930	— 29-226/8	Het C	F_2	*T. vulgare* × *T. compactum*	Gave Strain β 5
γ 3	Wellensiek 1939	— 39-10/13	"Gives 5 N : 4 H : 1 Sp"		—	None
γ 4*	Nilsson-Ehle 1927	26-1295 26-95/97	No information; apparently from same variety as β 4			
α/γ*	Åkerman 1926	1924-443 25-56/61	Het A "Constant A type"	Het	Schlanstedt × 0715	S. G. Smith (34)
α 1	Åkerman 1926	1926-493 26-74	Het A in 1926	F_8	Extrakolben × Br. Schlanstedt	Sib of β 2
α 2	Nilsson-Ehle 1929	— 29-27/30	Het A	N	*T. vulgare* var. Iron	None

* *Strains supplied in 1929 to Dr. Uchikawa.*

Symbols for chromosomes are: C = the chromosome which ordinarily carries on its long arm the genes which determine the "normal", *vulgare* type of glume and the tip-awned or "beardless" condition—it is chromosome IX of Sears (32); Cd = a C chromosome with a deficiency detectable at metaphase and thereby lacking the above genes; Ctl = the telokinetic long arm of the C chromosome; Cts = the telokinetic short arm of the C chromosome; C^{α} = the mutated C chromosome of Series α which has a deficiency too small to be established cytologically at metaphase but determinable genetically as about 30 cross-over units in length; Cil = the isochromosome formed by the duplication of the long arm of a normal C chromosome—it is the "Co" or compactoid-forming, "secondary" chromosome of Hakansson (6) and is, of course, equivalent to two Ctl chromosomes in gene content.

III. Observations

A. Speltoids of Series β

(*a*) Strain β 1 (Fig. 23)

Three progeny of Dr. Åkerman's Het Sp 1924-440 r4 were collected by C. L. Huskins at Svalöf in 1926. One was a normal which bred true. The other two were classified as het speltoids. One (26-55) gave a fairly typical Series β progeny ratio as did its Het Sp progeny: 91 N, 414 H, 6 Sp, and 1 unclassified dwarf (Table II).

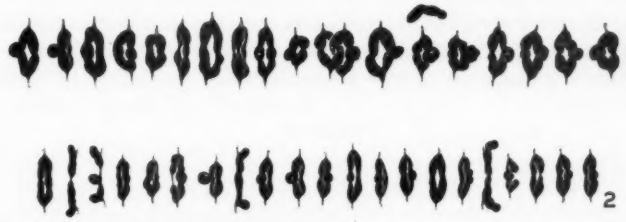
TABLE II

Parent plant	Year grown	Generation		No. of families	No. of seeds		Progeny				
		No.	Sown		Sown	Germ.	N	H	Sp	Dwf.	Total
1 Original H 26-55	1926	D1	1927	1 —	43	39	8	26	0	0	34
2 H ex H	1927	D2	1928	7 —	396	327	57	255	4	0	316
3 Resowing	—	—	1930	— 1	3	3	1	2	0	0	3
4 H ex H	1928	D3	1929	3 —	153	109	18	88	2	0	108
5 H ex H	1930	—	1934	1 —	12	12	2	9	0	0	11
6 H ex H	1928	—	1937	1 —	60	26	3	23	0	0	26
7 H ex H	1930	—	1937	1 —	36	17	2	11	0	1	14
8 Total	—	—	—	14 1	703	533	91	414	6	1	512
9 Original N 26-53	1926	D1	1927	1 —	23	22	22	0	0	0	22
10 Total N progeny	'26, '27	D1, 2	'27, '28	5 —	71	63	63	0	0	0	63

The other produced a very varied and, in general, much more vigorous progeny. Both from its segregation and its cytology Huskins (9) concluded that it must have been a hybrid between *T. vulgare* and *T. spelta* instead of a Het Sp. This conclusion was later confirmed by Dr. Åkerman. It evidently arose through fertilization of a normal ovule of Het Sp 1924-440 r4 by a pollen grain from one of the segregates of a cross with *T. spelta* which were being grown in adjacent plots at Svalöf by Dr. Nilsson Leissner. Plant 26-54 and its progeny will not therefore be considered further in the present analysis of Strain β 1. Natural crossing between it and a strain intermediate between Series α and γ gave other complications (34).

In three het speltoids of strain β 1 Huskins (9) found a total complement of 41 chromosomes which were "arranged regularly as $20_{II} + 1_I$" in pollen mother cell meiosis. Two further het speltoid progeny have since been examined in smear preparations, with the same result (Fig. 1) except that out of many hundreds examined two cells were found with $19_{II} + 3_I$. The metaphase shape of the univalent "C" chromosome in this strain has been taken as standard since it seems to be characteristic for most of the β and γ

Series strains that we have examined. The univalent C chromosome is divided by the kinomere into two arms, one about four times the length of the other, and the longer arm bears a secondary constriction which divides it



FIGS. 1, 2. *Segregates of strain β 1:*
1. *Metaphase I, het speltoid;* $20_{II} + C_I$. *Note two bivalents interlocked.*
2. *Normal segregate with* 21_{II}.

into a proximal and distal segment of relative lengths 3 : 5. It is usually found lying either at the periphery of the plate or just off it at metaphase I. As the members of the bivalents pass towards the poles at early anaphase the univalent moves on to the plate. When the remainder of the chromosomes are nearing late anaphase the univalent "splits" and its longitudinal halves proceed to opposite poles but very frequently arrive too late to be included in the daughter nuclei. Deviations from this behavior such as the inclusion of a complete ("unsplit") univalent or of the two halves of a univalent in one dyad nucleus have not been seen in this strain and only rarely in others though genetic evidence that it occurs occasionally is supplied by the occurrence of Type II subcompactoid segregates with three C chromosomes from het speltoid parents having only one (35). Nor has transverse fragmentation of the univalent been seen in this strain but there is evidence, in the regular origin of CCil subcompactoids from β het speltoids, that it occurs not infrequently and Love (19) has observed it in other wheat mutants. During the second division the "daughter univalents" may be left out of the microspore nuclei or may be included without further "splitting".

Since $20_{II} + 1_I$ are formed so regularly in β het speltoids, the proportion of pollen grains with 20 and 21 chromosomes may be estimated from counts made of micronuclei lying outside the pollen tetrad nuclei. From 150 pollen tetrads it was calculated that 86% of the pollen grains contain only 20 chromosomes – see Section IV.

One normal segregate of strain β 1 was found by Huskins (9) to have 21_{II}. One other normal examined since in smear preparations also regularly has 21_{II} (Fig. 2) though out of hundreds of cells examined one was found with $20_{II} + 2_I$.

No satisfactory cytological material was obtained from the six dwarf speltoid segregates. They would, of course, be expected to have only 40 chromosomes, and we found this in a β speltoid derived from a subcompactoid

and repeatedly in the analogous fatuoid segregates. It has also been found by Vasiliev (37), Uchikawa (36), Sears (32), and others.

(*b*) Strain β 2 (Fig. 24)

A second Series β strain (Table III) has been derived from a single newly arisen het speltoid plant, 26-72, found by C. L. Huskins in 1926 in one of Dr. Åkerman's wheat plots. This plot of selected stock (his 1926-497) was

TABLE III

Parent plant	Year grown	Generation		No. of families	No. of seeds		Progeny				
		No.	Sown		Sown	Germ.	N	H	Sp	Other	Total
1 Original H 26-72	1926	D1	1927	1 —	49	46	14	27	0	0	41
2 H ex H	1927	D2	1928	3 —	108	90	15	74	0	0	89
3 Resowing	—	—	1935	— 2	48	24	4	14	0	0	18
4 H ex H	1935	D3	1936	3 —	105	43	14	26	1	1*	42
5 H ex H	1936	D4	1937	1 —	24	10	1	6	0	0	7
6 Total	—	—	—	8 2	334	213	48	147	1	1*	197
7 N ex H	1927	D2	1928	1 —	12	11	11	0	0	0	11
8 Original chimera 26-73	1926	D1	1927	1 —	22	21	21	0	0	0	21
9 Short-bearded H	1936	D4	1937	1 —	12	5	0	0	0	2*	2

* *Short-bearded H due to natural crossing.*

an F_9 progeny from the cross of two standard varieties of *T. vulgare*, Extra-kolben × Braun Schlanstedt. The short-bearded het speltoid segregate of D_3 had $20_{II} + 1_I$ but the distal part of the C chromosome seemed to be somewhat broader than in its sib het speltoids and resembled that of strain β 4 (Fig. 3 and Fig. 34). Most probably this plant arose through fertilization

FIG. 3. *Hybrid het speltoid from* β *2;* $20_{II} + C_I$ (4_{II} *interlocked*).

by a normal pollen grain from one of our Nilsson-Ehle strains, growing in an adjacent bed, in which both the normals and het speltoids are partly bearded.

The Strain β 2 het speltoids varied in the shape and tightness of the glumes. Either the parental F_9 selection stock had not yet become homozygous or there had been natural crossing before or after the het speltoid plant was obtained from it.

Five beardless het speltoids all had $20_{II} + C_I$ and a normal sib had 21_{II}. Cytological material could not be obtained from the single dwarf, sterile, speltoid segregate.

A sib of the original het speltoid, collected at the same time, had only one culm and this was completely het speltoid on one side of the spike but normal in the lower half and het speltoid in the upper half of the other side. Twenty-two seeds from this spike produced 21 normal progeny. The plant was therefore only superficially a chimera.

(*c*) Strain β 3 (Figs. 25 and 26)

This is a winter wheat which has not been grown satisfactorily in Quebec. Progeny ratios and cytological material were obtained from three generations grown in England in 1927-9. Åkerman (2) has described its origin as a chimera occurring in 1919 in the progeny of a het speltoid out of a South Swedish variety. Three het speltoid sibs of the chimera which he propagated from his 1921-924 strain, gave 102 N, 611 H, and 4 weak, sterile Sp progeny. In the next generation, his 1924-921, 10 het speltoids gave 85 N, 334 H, and no speltoid progeny. Of present interest is the occurrence of a fertile and vigorous speltoid among the immediate progeny of the chimera.

One normal, three het speltoids, and one speltoid of this strain were obtained from Dr. Åkerman. The speltoid was a descendant of the vigorous speltoid from the chimera. Sixteen het speltoids have in three generations (Table IV)

TABLE IV

Parent plant	Year grown	Generation		No. of families	No. of seeds		Progeny				
		No.	Sown		Sown	Germ.	N	H	Speltoids		Total
									Fert.	Ster.	
1 Original H 26-69/71	1926	D1	1927	3	84	45	8	32	0	0	40
2 H ex H	1927	D2	1928	8	223	217	28	181	2	1	212
3 H ex H	1928	D3	1929	5	240	207	24	106	0	0	130
4 Total	—	—	—	16	547	469	60	319*	2	1	382
5 Original N 26-68	1926	D1	1927	1	20	13	13	0	0	0	13
6 N ex N	1927	D2	1928	1	36	34	29	0	0	0	29
7 Total	—	—	—	2	56	47	42	0	0	0	42
8 Original Sp 26-67	1926	D1	1927	1	30	10	0	0	7	3	10
9 Total Sp progeny	'26-'28	D1-3	'27-'29	8	222	187	0	4	166	7	177
10 N ex H	1927	D2	1928	1	36	33	33	0	0	0	33
11 Total N progeny	'27, '28	D2, 3	'28, '29	2	48	44	43	0	0	0	43
12 Sp ex H	1928	D3	1929	1	36	27	0	0	11	1	12

* *Including one H-N chimera.*

given us 60 N, 318 H, 1 chimera, and 3 Sp progeny. The chimera plant bore one chimerical (Fig. 25), two normal, and three het speltoid heads. The three speltoids all occurred in one year, 1928, in a family in which the germination and survival rate was particularly high. One of them was dwarf and sterile, one was of medium height, and one of nearly the same height as the normal sibs. The one of medium height had shorter beards than typical bearded speltoids and produced only a few seeds. Thirty-six seeds from the tallest one produced 1 sterile and 11 fertile speltoids.

Four het speltoids were examined by Huskins (9) and all found to have a typical β complement of $20_{II} + C_I$. Smear preparations confirm this.

Four normal plants bred true. Two of them examined by Huskins (9) had 21_{II} excepting for two pollen mother cells, out of about 350 observed, which were thought to contain trivalents. These would probably now be interpreted as bivalents with an unusually large segment distal to unterminalized chiasmata. One apparently true-breeding segregate of normal phenotype examined in a smear preparation made from paraffin material has $21_{II} + 2$ fragments. The speltoid out of the vigorous fertile speltoid from Åkerman's chimera, together with its descendants produced 4 H, 166 fertile Sp, and 7 sterile Sp progeny. The four het speltoids were almost certainly natural crosses. They gave 6 N, 34 H, and 1 dwarf, sterile Sp; they were Series β as would be expected if they resulted from ovules lacking a C chromosome being cross-fertilized by normal pollen. Åkerman (2) found two such putative hybrids among the 44 progeny of the original speltoid from the chimera.

In one of the speltoids grown in 1927 (D_1 from the speltoid obtained from Åkerman) Huskins (8, 9) recorded the occurrence of 41 chromosomes. He reported, however, that the odd chromosome was not regularly unpaired, as it is in 41-chromosome het speltoids. Trivalents of various shapes were reported to occur in a number of cells. In one cell there were three univalents. From paraffin material of this plant a smear preparation containing 10 good cells has been obtained. The complement is now found to be 41 chromosomes and two fragments. The two fragments were paired in three cells (Fig. 4)

FIG. 4. *Vigorous speltoid from β 3; $20_{II} + 1_I + f_{II}$ (f_{II} = paired fragments). Note high frequency of interlocking (dissolved paraffin preparation).*

and associated with a bivalent in one; they could not be seen in four and may therefore have been associated with other chromosomes in these cells. In one cell at metaphase, the two fragments and the univalent were all unassociated. In another at anaphase the univalent and one fragment were splitting belatedly—the other fragment had apparently been paired with a bivalent from which it had already disjoined. Re-examination of the original slides of sectioned material has shown that the "three univalents" in Huskins' (9) Fig. 26 are the univalent and the two fragments unassociated. Some of

the "trivalents" noted in the early records can, after re-examination, still be so interpreted but we hesitate now to make definite decisions about many of the apparently multivalent configurations seen in sectioned material.

(*d*) Strain β 4 (Fig. 27)

This is Nilsson-Ehle's strain 26-1292 of which three het speltoids were obtained in 1926 and given the numbers 26-92/94. The normal and het speltoid segregates are both short-bearded, but there is much variation in number and length of beards in both types. The sterile dwarf speltoid segregates are fully bearded. Twenty-six het speltoids of this strain gave a total of 175 N, 816 H, 3 Sp, and 1 SC progeny besides two dwarfs that did not head (Table V). Five normals gave 160 N and 1 H progeny, the latter an apparent natural cross.

TABLE V

Parent plant	Year grown	Generation		No. of families	No. of seeds		Progeny				
		No.	Sown		Sown	Germ.	N	H	Sp	Others	Total
1 Original H 26-92/4	1926	D1	1927	3 —	150	121	22	77	0	0	99
2 H ex H	1927	D2	1928	12 —	444	393	59	330	0	0	389
3 Resowing	—	—	1930	— 1	3	3	0	2	0	1 SC	3
4 H ex H	1928	D3	1929	5 —	275	230	49	181	0	1 dwf.	231
5 H ex H	1929	D4	1930	6 —	288	279	45	226	3	1 dwf.	275
6 Total	—	—	—	26 1	1160	1026	175	816	3	3	997
7 N ex H	1927	D2	1928	2 —	24	22	21	1	0	0	22
8 Total N progeny	'27, '29	D2, 3	'28, '30	5 1	169	161	160	1	0	0	161

One of the het speltoids in D_2 was superficially a chimera. It bore three het speltoid heads and one that was normal on one side and het speltoid on the other. The seeds of this head were all numbered according to their position and planted separately following the system of Åkerman (2). There was no correlation between glume type and the genetic constitution of the seeds; the normal side of the head gave the same segregation as the rest of the plant.

The chromosome constitution of the subcompactoid sib was $19_{II} + 1_I + C_I + Cil$, which is the same as that of a Type I subcompactoid except that one unidentified chromosome was lacking.

Six het speltoid plants of this strain were found by Huskins (9) to have $20_{II} + C_I$. Four more since studied in smear preparations have the same. In these acetocarmine preparations, however, part of the univalent distal to the secondary constriction often appears to be a little broader at metaphase than in the preceding three strains, see Fig. 5.

Counts of micronuclei were made in 150 het speltoid pollen tetrads. They indicate that 83.5% of the pollen grains contain only 20 chromosomes.

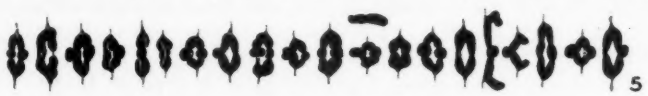


FIG. 5. *β 4 het speltoid; $20_{II} + C_I$.*

Two normal segregates examined by Huskins (9) and one examined since had 21_{II}. The three dwarf speltoid segregates were all too mature for cytological study when first identifiable. They were sterile. Three of the D_2 het speltoids were sent to Dr. Uchikawa, who obtained in four years a total of 374 N, 1750 H, 28 Sp, 9 SC, 5 "Short compactum", and 3 "Dwarf compactum" (36). He found 20_{II} in most p.m.c. of the speltoids, but some univalents and multiple associations. They were reported to be about 50% fertile—but see later.

B. Speltoids of Series γ

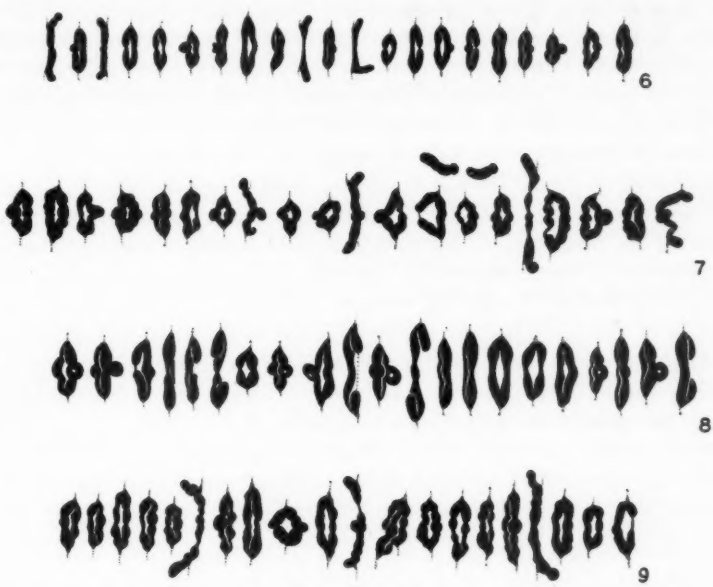
(*a*) Strain γ 1 (Fig. 29)

This was Åkerman's 1924-444 when two speltoids (26-62/3) and three het speltoids (26-64/6) were obtained from him in 1926. On the basis of Nilsson-Ehle's definition he had originally classified it as Series A rather than C because it did not produce more normals than het speltoids. It has given us (Table VI) 161 N, 207 H, and 13 Sp and we class it as Series γ.

TABLE VI

	Parent plant	Year grown	Generation		No. of families		No. of seeds		Progeny			
			No.	Sown			Sown	Germ.	N	H	Sp	Total
1	Original H 26-64/6	1926	D1	1927	3	—	120	92	40	48	2	90
2	H ex H	1927	D2	1928	4	—	132	97	40	54	2	96
3	Resowing	—	—	1930	—	1	4	4	2	2	0	4
4	Resowing	—	—	1935	—	1	48	30	9	16	0	25
5	H ex H	1928	D3	1929	3	—	192	167	65	81	8	154
6	H ex H	1930	—	1936	1	—	36	12	5	6	1	12
7	Total	—	—	—	11	2	532	402	161	207	13	381
8	Original Sp 26-62/63	1926	D1	1927	2	—	60	29	0	1	26	27
9	Total Sp progeny	'26, '27	D1, 2	'27, '28	9	—	203	143	0	1	136	137
10	N ex H	1927	D2	1928	9	—	180	153	152	0	0	152
11	Sp ex H	1927	D2	1928	1	—	24	20	0	0	20	20
12	Total Sp progeny	'27-'30	D2, 3	'28-'36	3	1	61	43	0	1	41	42

Five het speltoids had 21_{II} in most p.m.c. nuclei, but one of these bivalents was heteromorphic (Fig. 8). In a sample of 50 cells the heteromorphic pair were unassociated in six (Fig. 9). The larger member when unassociated is



FIGS. 6 to 9. *Segregates of strain γ 1:*
6. *Het spelt oid with* $20_{II} + CCd_{II}$.
7. *The same with C and Cd unpaired.*
8. *Normal with* 21_{II}.
9. *Spelt oid with* 21_{II} *(two bivalents interlocked).*

similar in form to the univalent C chromosome of β het speltoids. The shorter member is only about three-quarters as long and the pairing relationships indicate a deficiency in that portion of the C chromosome major arm distal to the secondary constriction – see description of the heteromorphic pair in Strain γ 2 and Figs. 11 and 12 from it. When unassociated, these "C" and "C-deficient" ("Cd") chromosomes move on to the plate and "split" during the first anaphase (Fig. 36) as does the univalent in Series β. A sixth het speltoid had 20_{II} + C (Series β). It arose from a het speltoid known to have 20_{II} + C + Cd (Series γ). Three of its het speltoid sibs had the same constitution as the parent and a normal sib had 21_{II}.

One D_4 het speltoid obviously arose from natural crossing. It was brown and its progeny comprised 12 brown and six white N, 19 brown and seven white H, and two brown Sp. They varied in length of awns. One of these het speltoid progeny had a typical Series γ chromosome complement, but another had a Series β complement.

Three normals, two examined by Huskins (9) and one since, all bred true and have normal chromosome number and behavior (Fig. 8).

The two speltoids obtained from Åkerman and their speltoid progeny gave 136 Sp and 1 H. Three speltoids from het speltoids gave 41 Sp and 1 H progeny. The first het speltoid gave 18 N and 17 H progeny and segregated for glume color; it therefore arose from pollination by a normal gamete from a different strain. It should be stressed that no well-established case (i.e. one in which the possibilities of natural crossing or seed admixture are ruled out) is known of reversion from speltoid to het speltoid or normal.

A speltoid of this strain had 21 homomorphic bivalents (Fig. 9). Presumably one of these is a pair of Cd chromosomes but they cannot be identified when all the bivalents are homomorphic. The shorter length of the Cd is obvious when it is accompanied by a normal C chromosome in the het speltoids, but a CdCd bivalent in a speltoid is no smaller than some of the other bivalents.

Some of the progenies from these speltoids were weak and more or less sterile plants while others were little different from het speltoids of this strain in vigor and fertility. It can never be taken for granted that speltoids from β or γ het speltoids or speltoids are themselves of the parental series, for both natural crossing and secondary chromosome aberrations are common and either may have entered into their origin. In the present case it seems probable that the weaker speltoid progenies were true Series γ and the others not.

(*b*) Strain γ 2 (Fig. 31)

This is the strain which Philiptschenko (29) reported as giving a γ progeny ratio while having a β chromosome constitution. The cytological study cited by Philiptschenko was carried out by Vasiliev (37) who found 21_{II} in pollen mother cells of normal plants, $20_{II} + 1_{I}$ in het speltoids and 40 chromosomes in root tip cells of a speltoid segregate.

The strain originated from a het speltoid found by Philiptschenko in an F_2 of *T. compactum creticum* × *T. vulgare* var. Marquis. In 1927 (F_3 of the hybrid family) the original het speltoid gave him 20 N and 25 H progeny. In 1928 he sowed 450 seeds from six normal plants, but owing to unfavorable weather conditions obtained only 204 mature plants. All were normals. From eight het speltoid plants 620 seeds were sown, but only about 200 progeny grew and many were badly developed. Only 174 could be classified; these consisted of 68 N, 100 H, and six Bearded Sp. Detailed descriptions and measurements of all three types are presented by Philiptschenko and the differential mortality of het speltoid progeny is discussed. For present purposes we need cite only the fact that five speltoid culms were just slightly taller than 80 culms of their het speltoid sibs. Presumably each culm represented a different plant, but this is not specifically stated. In view of our later findings, it is important to note that in any case at least one speltoid segregate was not measured. This may have been the one used for cytological

study and found to have only 40 chromosomes. We have never, either in wheat or oats, found 40-chromosome plants taller than their 41- or 42-chromosome sibs.

In 1929 heads of three het speltoid plants (probably F_4) were obtained from Professor Philiptschenko. They were more like *T. spelta* than any of our other speltoid strains excepting those plants of the α/γ strain (Smith, unpublished) which almost surely had a *T. spelta* ancestor. Seeds of the three het speltoids were sown in 1930 under the numbers 29-226/228 (Table VII).

TABLE VII

	Parent plant	Year grown	Generation		No. of families		No. of seeds		Progeny			
			No.	Sown			Sown	Germ.	N	H	Sp	Total
1	Original H 29-226/228	1929	D1*	1930	3	—	78	75	33	41	1	75
2	Resowing of 29-228			1935	—	1	36	25	10	11	0	21
3	γ H segregates	1930	D2	1931	5	—	120	95	50	41	3	94
4	Resowings			1932	—	3	72	50	19	30	0	49
5	Resowing			1935	—	1	36	12	7	5	0	12
6	Resowing			1936	—	1	48	11	4	4	0	8
7	β H segregates ex γ			1931	3	—	72	54	7	44	1	52
8	γ H segregates	1931	D3	1932	8	—	192	170	73	90	3	166
9	Resowings			1934	—	3	72	53	28	21	1	50
10	β H segregates ex β			1932	3	—	72	61	10	50	1	61
11	β H segregates ex β			1934	1	—	30	26	3	23	0	26
12	γ H segregates			1935	3	—	132	107	43	54	2	99
13	Resowing			1937	—	1	48	12	5	7	0	12
14	Total for Series γ	—	—	—	19	10	834	610	272	304	10	586
15	Total for Series β 5	—	—	—	7	—	174	141	20	117	2	139
16	N segregate ex γ H	1930	D2	1931	1	—	24	23	23	0	0	23
17	Total N progeny	'30, '31	D2, 3	'31, '32	2	—	48	46	45	0	0	45
18	Sp ex γ H	1930	D2	1931	1	—	12	7	0	0	4	4
19	Total Sp progeny	'30-'34	D2-4	'31-'35	4	—	38	24	0	0	19	19

* *The first generation from seeds obtained from Professor Philiptschenko; probably F_5 of his cultures.*

The D_1 progenies (though small) indicate that the parents probably belonged to Series γ and cytological analysis supports this view. Two phenotypically similar D_1 het speltoids from plant 29-226 were examined. One was a typical Series γ having 42 chromosomes including a heteromorphic pair; the other was a β type having only 41 chromosomes. In D_2 the first plant gave a γ progeny ratio; the second plant gave a β ratio. Similarly from D_1 family 29-227 one het speltoid was found to have 42 chromosomes including a heteromorphic pair and another had only 41. Again the progeny ratios followed

cytological expectation. From D_1 family 29-228 the one normal plant examined had 21_{II}, three het speltoids had 20_{II} + CCd, and one het speltoid had $20_{II} + C_I$. The normal bred true, the two CCd het speltoids tested gave γ ratios, and the $20_{II} + C_I$ plant gave a β ratio. One het speltoid from family 29-228 which was not examined cytologically gave a Series γ ratio. The one speltoid segregate tested bred true.

In succeeding generations het speltoids of the β Series (Strain β 5) continued to give β ratios, while γ het speltoids continued to give both β and γ het speltoid progeny. Cytological examinations or genetic tests, or both, were made of 36 het speltoid progeny of γ het speltoids. Of these, 31 were γ and five were β.

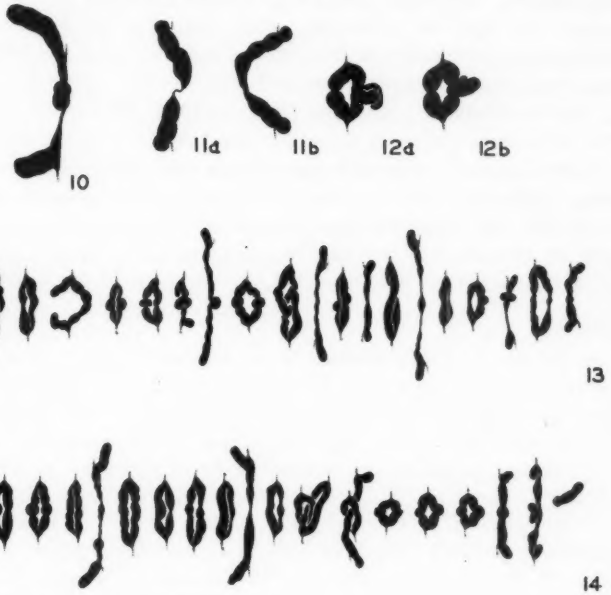
Four normals all had 21 homomorphic pairs. Nine het speltoids which gave β ratios all had 41 chromosomes. Four of them were derived directly from γ het speltoids and five were from β het speltoid segregates. They usually had $20_{II} + 1_I$ but a few cells were seen with $19_{II} + 3_I$. Observations on 150 first division telophases and 150 pollen tetrads indicate that the majority of male gametes formed have only 20 chromosomes. That very few of these function in fertilization is shown by the total segregation of 20 N, 117 H, and only 2 Sp (Table VII).

One β het speltoid segregate had 42 chromosomes, as had also three of its het speltoid descendants, but these never formed 21_{II}. Characteristically there were 19_{II}, a C_I, and a non-C trivalent. The non-C trivalent was also found in fertile speltoid segregates (Fig. 40). There is evidently the usual unpaired C chromosome and an extra chromosome other than C involved in this case. This chromosome appears to compensate for the lack of a C in that it apparently increases the functioning capacity of speltoid-determining pollen grains. The ratio from this het speltoid and three similar progeny was 21 N : 111 H : 21 Sp, which is rather like the "modified β" ratio found in fatuoids.

In the γ het speltoids of this strain the CCd chromosomes are rather frequently unassociated at metaphase. In a sample of 154 p.m.c. they were associated in 133 (Fig. 37) and unassociated in 21 (Fig. 35). Very occasionally two pairs were unassociated; it could not be determined whether the second pair was the same in all cases.

It seems evident that, as in the previous strain, the difference in size between the members of the heteromorphic pair of γ het speltoids is due to an interstitial deficiency in one of them. The size difference is considerable (about 26%); therefore were it due to translocation, associations should sometimes be formed with other chromosomes and none was observed. Likewise, were it due to a duplication in the larger member this might sometimes form a loop or ring within itself and this was not seen. The larger member appears identical in form with the univalent of β het speltoids of this and other strains, which together with the genetic data supports the deficiency interpretation.

That it is an interstitial deficiency is indicated by the fact that the pair can be associated terminally by either the short arms (Fig. 10) or the unequal long arms (Figs. 11*a* and 11*b*). That it is situated in the distal half of the



FIGS. 10 to 14. *Segregates of strain γ 2:*

10. *C and Cd united by their short arms.*
11. *C and Cd united by their long arms—one normal and the other segmentally deficient.*
12. *C and Cd united by both arms.*
13. *Speltoid with 21_{II} (two complete interlocks).*
14. *Speltoid with $20_{II} + Cd_{I}$ (one complete and one incomplete interlock).*

major arm is indicated both by its shape and by the fact that where associations occur like those in Figs. 12*a* and *b*, in which a chiasma is present about halfway along the longer member, the regions distal to it are of unequal length.

When C and Cd are unassociated at first metaphase they often fail to become included in the first or second division nuclei. By the recombination of 20 + Cd and 20-chromosome gametes, speltoids of three chromosomal types, 40 + 2 Cd, 40 + Cd, and 40, will be expected in the progeny of γ het speltoids but the speltoids segregated by β het speltoids should all be of the 40-chromosome type. The 40-chromosome type was found by Vasiliev (37) but it is not certain whether it was the progeny of a β or γ het speltoid. We have obtained the other two types, though from a speltoid segregate of 29-228 and not directly from a het speltoid; they are phenotypically alike. The 42-chromosome speltoid usually had 21_{II} (Fig. 13) but there was a high

frequency of interlocking at metaphase. Two speltoids with 41 chromosomes usually had $20_{II} + 1_{I}$ (Fig. 14). The behavior of the univalent, which from its shape seemed to be a Cd chromosome, was like that of the C chromosome in β het speltoids. Speltoids of this type should, therefore, produce two types of gametes, one with 20 + Cd, the other with only 20 chromosomes and, apart from natural crossing, their offspring should all be speltoids, as eight progeny from one such plant were found to be. The speltoids with 20_{II} + CdCd and those with only 20_{II} apparently differ only in vigor and fertility.

The high frequency, 14%, with which β het speltoids arise from γ in this strain was evidently responsible for the difficulty Philiptschenko experienced in his analysis. To harmonize his ratio with our joint β and γ ratios it is only necessary to assume that two of the eight het speltoids he tested were β segregates, as was the speltoid in which Vasiliev counted 40 chromosomes.

(*c*) Strain γ 3 (Fig. 28)

During the winter of 1939-40 two "compact ears" of wheat and two "long-ears" were received from Dr. S. J. Wellensiek. The "long-eared" plants of this strain were said to segregate "compact", "long", and "speltoid" in the ratio of approximately 5 : 4 : 1 and the "compact" to breed true. The long-eared type is not unlike the normals of some of our other strains in either glume shape or head laxity, but it is intermediate between the "compact" type and the speltoid. The "compact" type is not unlike some of the sub-compactoid mutations to be dealt with later, but has squarer glumes than any of them. As emphasized in (12), varieties of *T. vulgare* differ greatly with respect to the characteristics involved in the "speltoid complex" or its "normal" counterpart and the classification of the normal and het speltoid segregates from any given het speltoid can only be relative to the characteristics of the variety from which it arose. These "compact" and "long" ears are the "normal" and "het speltoid" type for this strain, though the latter has little resemblance either to ordinary speltoids or to *T. spelta*.

Three seeds from each normal ("compact") head and 12 from each het speltoid ("long") were sown immediately in the greenhouse. The normals gave normals and the het speltoids gave 6 N, 12 H, and 1 Sp. The speltoid was typical in having lax heads with full beards and sharply keeled, indurated glumes. It was taller than its normal and het speltoid sibs.

Two normals had 21_{II}. The het speltoids had either 20_{II} plus one heteromorphic pair (Fig. 15) or 20_{II} plus two univalents of different length.

FIG. 15. *γ 3 het speltoid; 20_{II} + CCd_{II}.*

When unpaired the two looked like the C and Cd chromosomes of other Series γ strains. A D_2 sowing made in the spring of 1942 to determine the genetic ratio failed to head; it is evidently a winter wheat.

(*d*) Strain γ 4 (Fig. 30)

Three plants classified under his numbers $26\text{-}1295_{1\text{-}3}$ as het speltoids of Series C were obtained in 1927 from Professor Nilsson-Ehle. Their progeny are recorded in Table VIII where it will be seen that the strain now comprises three types treated here as substrains, viz., (*a*) the het speltoids which continued segregating as Series γ; (*b*) het speltoids segregating as Series α; and (*c*) a het speltoid with extremely narrow glumes which segregated normals, het speltoids like itself, and speltoids, also with atypical narrow glumes; this form arose among the progeny of a normal segregate of substrain (*a*). In addition, the expected Series β arose from two D_2 het speltoids of substrain (*a*) which were given to Dr. I. Uchikawa (36).

Substrain (*a*)

This strain gave 230 N : 271 H : 1 Sp. Uchikawa (36) obtained from it 347 N, 380 H, and 21 Sp. This is a γ strain on our definition; though obtained directly from Professor Nilsson-Ehle as Series C it has not proved to be such on his definition which requires an excess of normals.

The segregated normals and their normal offspring gave 452 normals, three ordinary het speltoids, and the narrow-glumed het speltoid progenitor of substrain (*c*). One of the "normals" which gave a het speltoid had only 41 chromosomes (9) and the parents of the other two were dwarf plants and may therefore also have been chromosomally unbalanced. One of the het speltoids was sterile. One had brown glumes and was clearly a hybrid with another strain. That which arose from the 41-chromosome "normal" resembled other het speltoids of this strain. It gave 17 N and 13 H progeny.

Six normals of substrain (*a*) had 21_{II}. A seventh had 21_{II} plus a fragment, Fig. 16. The fragment was not always detectable at metaphase, probably because it was sometimes paired with one of the bivalents. This plant bred true for the "normal" phenotype and the only one of its progeny examined had 21_{II} and a fragment like that of its parent. The 41-chromosome "normal" which gave rise to the het speltoid gave in addition 64 normal progeny. Two of these were examined and each had 21_{II}. The chromosome missing from the parent plant was evidently not a C chromosome.

Huskins (9) reported that the het speltoids of this substrain had 43 chromosomes "probably usually arranged as $20_{II} + 1_{III}$". In one, however, he found "a further cytological abnormality in the loss of approximately half a chromosome." This was later, however, found to be the primary characteristic of these het speltoids and we now regularly find 21_{II} (Fig. 17) of which one is extremely heteromorphic. The smaller member is about as long as the short

TABLE VIII

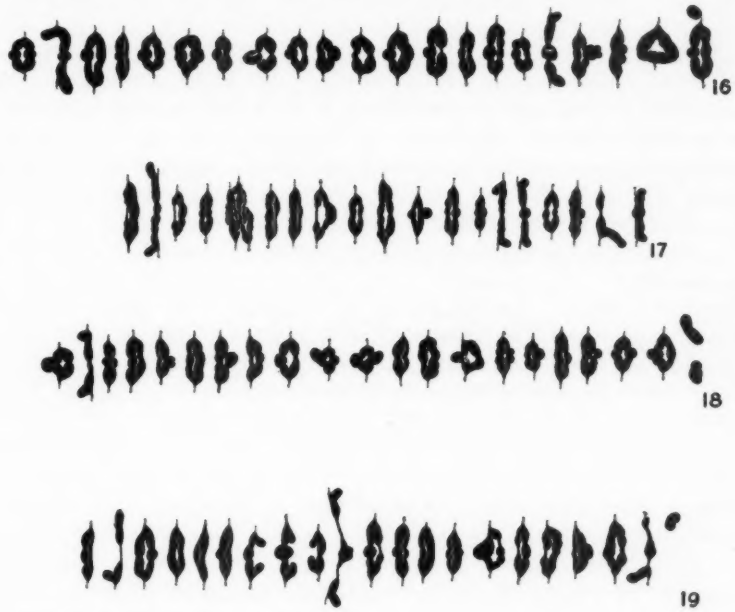
	Parent plant	Year grown	Generation		No. of families		No. of seeds		Progeny				
			No.	Sown			Sown	Germ.	N	H	Sp	Others	Total
(a)	1 Original H 26-95/97	1926	D1	1927	3	—	150	116	48	67	0	0	115
	2 Resowing 26-96	—	—	1935	—	1	24	11	7	1	0	0	8
	3 H ex H	1927	D2	1928	5	—	240	193	77	95	0	0	172
	4 H ex H	1935	—	1936	1	—	17	4	1	3	0	0	4
	5 H ex H	1928	D3	1929	6	—	252	207	97	105	1	1*	204
	6 Total	—	—	—	15	1	683	531	230	271	1	1*	503
	7 N ex H 26-97	1927	D2	1928	1	—	36	34	34	0	0	0	34
	8 N ex H 26-96	1935	—	1936	1	—	24	11	10	0	0	0	10
	9 41-chr. N ex H 26-95	1927	—	1928	1	—	72	65	64	1	0	0	65
	10 N ex H	1928	D3	1929	3	—	102	93	91	0	0	1**	92
	11 N ex 41-chr. N	—	—	—	3	—	70	63	36	0	0	0	36
	12 Dwf. N ex Dwf. N	1929	D4	1930	4	—	108	105	103	2	0	0	105
	13 Dwf. N ex Dwf. N	1930	D5	1931	2	—	48	46	43	0	0	0	43
	14 Superficial chimera	—	—	—	1	—	88	71	54	0	0	0	54
	15 Dwf. N ex Dwf. N	1931	D6	1932	2	—	24	19	17	0	0	0	17
	16 Total	—	—	—	18	—	572	507	452	3	0	1**	456
	17 H ex 41-chr. N	1928	D3	1929	1	—	36	31	17	13	0	0	30
(b)	18 Hybrid H ex 26-96	1927	D2	1928	1	—	36	31	8	14	7	0	29
	19 H ex hybrid H	1928	D3	1935	2	—	48	35	12	8	4	0	24
	20 Resowing	—	—	1936	—	1	48	6	3	1	0	0	4
	21 H ex H	1935	D4	1940	3	—	96	54	16	21	14	1†	52
	22 Total	—	—	—	6	1	228	126	39	44	25	1	109
	23 Sp ex hybrid H	1928	D3	1929	2	—	48	40	0	0	40	0	40
(c)	24 "H" ex N	1929	D4	1930	1	—	24	22	10	12	0	0	22
	25 "H" ex "H"	1930	D5	1931	1	—	24	23	11	5	1	0	17
	26 Resowing	—	—	1932	—	1	24	22	7	13	0	0	20
	27 "H" ex "H"	1931	D6	1932	2	—	48	43	14	26	0	0	40
	28 Total	—	—	—	4	1	120	110	42	56	1	0	99
	29 Sp ex "H"	1931	D6	1932	1	—	17	12	0	0	12	0	12
	30 Total Sp progeny	'31, '32	D6, 7	'32, '36	2	—	29	17	0	0	17	0	17

* *A natural hybrid which gave 54 N : 17 H and a superficial N – H chimera, which gave only normal progeny, see line 14.*

** = "*H*", *a het speltoid with shoulderless and narrow, but typically indurated glumes, see l. 24.*

† *Dwarf.*

arm of a normal C chromosome and is telokinetic; we designate it Cts. It is probable that the three segments of the heteromorphic bivalent were taken to be whole chromosomes in the original counts of 43. The initial correct interpre-



FIGS. 16 to 19. *Segregates of strain γ 4:*

16. *"Normal" with 21_{II} + 1 fragment.*
17. *γ het speltoid with 20_{II} + $CCts_{II}$ (three bivalents interlocked).*
18. *"Normal" from narrow-glumed het speltoid; 20_{II} plus heteromorphic pair, here unassociated.*
19. *Narrow-glumed het speltoid; 19_{II} + $CCts_{II}$ + 1_{I}.*

tation of loss was made from cells in which C and Cts were unpaired. We have now found them unpaired in six out of a sample of 100 cells. Uchikawa (36) also found 20_{II} plus an extremely heteromorphic pair in het speltoid progeny of the plants he received from us. However, in the 3617 cells he examined, he found only about 0.4% with the heteromorphics unpaired. The difference may be due to environmental factors affecting meiosis or may in part be due to the fact that his material, like that of Huskins' original study, was sectioned.

One het speltoid had 19 apparently normal bivalents, the heteromorphic CCts, and a pair of fragments; it bore 32 seeds but from these only three mature progeny, all normals, were obtained.

Only one speltoid arose in this substrain. It was fully bearded and had only one culm. Uchikawa found the expected 20_{II} plus a very small pair, apparently CtsCts, in his speltoids of this strain.

Substrain (*b*)

In the D_1 generation of substrain (*a*), one short-bearded het speltoid arose which gave 8 short-bearded N, 14 short-bearded H, and seven fully bearded Sp progeny (Table VIII). The segregation from later generations was 31 N, 30 H, 18 Sp, and 1 dwarf plant which failed to head and hence could not be classified. The grouped ratio, 39 N, 44 H, and 25 Sp is not significantly different from that of some α strains. It is quite different from that of the parent strain. It appears that this substrain arose from crossing, while still in Nilsson-Ehle's cultures, with either *T. spelta*, or more probably, with a descendant of crosses between *T. vulgare* and *T. spelta* made earlier by Nilsson-Leissner (26) at Svalöf—the same hybrid material that contaminated our strain β 1 before it was obtained from Dr. Åkerman.

Two "het spelta-speltoids" from the D_1 of this hybrid had 21_{II} of which one appeared slightly heteromorphic. The glume type ranged from typical speltoid to typical *T. spelta*, indicating the existence of modifiers segregating independently of the C chromosome in this hybrid. These could, of course, be (or include) alleles (in the phylogenetic sense) of the spelta–speltoid gene series situated on homoeologous chromosomes (B or A of Winge). The heteromorphic pair of this substrain may have originated as a C chromosome from *T. vulgare* and a C^s chromosome from *T. spelta*. This would account both for the phenotype and the relative fertility of both types of pollen.

Meiotic irregularities similar to those found by Huskins (9) in the spelta hybrid of strain β 1 were found in all three of the main types of segregates. Three normals had 21_{II}; one had 20 homomorphic bivalents and a pair which appeared to be slightly heteromorphic; another had 20 normal bivalents plus a telokinetic pair; a sixth had 20_{II} and a small univalent. Six het speltoids all had one slightly heteromorphic bivalent. Accompanying it, one had the usual 20_{II}; one had 19_{II} plus two small chromosomes usually unpaired but possibly associated in four of the 109 nuclei examined; another had 19_{II} plus a second heteromorphic pair of which the shorter member was telokinetic; two had $19_{II} + 1_{III}$; another had 19_{II}, 1_{III}, and a pair of fragments. The only "speltoid" segregate examined had 21 homomorphic bivalents.

Substrain (*c*)

A plant with extremely narrow and shoulderless, though typically indurated and keeled, glumes arose in the D_3 generation of substrain (*a*) together with 27 normals as the progeny of a normal descendant of a het speltoid. Despite its atypical glumes it must be classified as a γ het speltoid (Table VIII). In later generations there was much sterility and many plants had badly formed heads which could not be classified. The genetic record is given only for the early generations. The normals examined had either 21_{II} or 20_{II} plus a heteromorphic pair (Fig. 18). Four constitutions were found in het speltoids: 20_{II} + CCts; 19_{II} + CCts plus one slightly heteromorphic pair; 19_{II} + CCts + 1_I and 19_{II} + CCts plus one pair of fragments. Apparently the primary

segregation was due to the CCts pair of the parent stock. The single speltoid examined had 21_{II} with much interlocking.

C. Speltoids of Series α

(*a*) Strain α 1 (Fig. 32)

This strain is derived from a single het speltoid obtained from Dr. Åkerman. It arose in 1926 in the F_9 of a cross Extra-Kolben × Schlanstedt. Its progeny ratio, 55 : 95 : 32 (Table IX), is typical for Series α. Two speltoid segregates gave 41 speltoids and one het speltoid which was a natural hybrid.

TABLE IX

	Parent plant	Year grown	Generation		No. of families		No. of seeds		Progeny			
			No.	Sown			Sown	Germ.	N	H	Sp	Total
1	Original H 26-74	1926	D1	1927	1	—	50	44	14	21	9	44
2	H ex H	1927	D2	1928	2	—	72	64	20	34	10	64
3	Resowing	—	—	1930	—	1	3	3	2	1	0	3
4	Resowing	—	—	1934	—	1	12	8	0	8	0	8
5	H ex H	1930	D3	1934	1	—	12	12	3	6	3	12
6	H ex H	—	—	1936	1	—	12	10	3	5	2	10
7	H ex H	1934	—	1935	1	—	48	39	7	16	7	30
8	H ex H	1936	D4	1937	1	—	12	6	3	2	1	6
9	Resowing	—	—	1938	—	1	24	10	3	2	0	5
10	Total of H	—	—	—	7	3	245	196	55	95	32	182
11	N ex H	1927	D2	1928	1	—	12	12	12	0	0	12
12	Sp ex H	1927	D2	1928	1	—	24	22	0	1	21	22
13	Total Sp progeny	'27, '30	D2, 3	'28-'36	2	2	49	42	0	1	41	42

Huskins (9) examined one plant from each class of segregates and reported 42 chromosomes in each. In the normal he found 21_{II}. Multivalents were recorded in the het speltoid and speltoid. There is no doubt that the quadrivalent shown in his Fig. 4 and the univalent in Fig. 2 are correctly interpreted, but Figs. 1, 2, and 3 are now considered doubtful interpretations. In smears we have found interlocked bivalents but no multivalents. Uchikawa (36) found quadrivalents in about 0.3% of the nuclei of all three classes of segregates, but there is a possibility that his material had a hybrid origin (mith, unpublished). It is now clear from Uchikawa's and our own work that trivalent formation is not a special characteristic of α het speltoids, nor quadrivalent formation of its speltoids. Typical metaphase plates of these het speltoids and speltoids are shown in Figs. 20 and 21.

The bivalents always appear to be composed of homomorphic pairs in the normals and speltoids and usually in the het speltoids also. When a bivalent associated at only one end is present in the het speltoids it sometimes appears

FIGS. 20, 21. *Segregates of strain α 1:*
20. *Het speltoid with* 21_{II}.
21. *Speltoid segregate with* 21_{II} *(two complete interlocks).*

to comprise a heteromorphic pair. The degree of inequality, however, is so slight that it is difficult to be certain that it is not due to unequal stretching or contraction. As a possible, indirect, test for heteromorphism due to deficiency, pollen tetrads of normals and het speltoids of this strain were compared for the presence of micronuclei. There were 19 in 1727 tetrads from normals and four in 272 from het speltoids. This is 1.10 and 1.46%, respectively and the difference is not significant. If there is a deficiency or any other structural change in one of the C chromosomes of these α het speltoids it evidently does not affect synapsis of the normal and mutated C chromosomes sufficiently to upset their association and disjunction and thereby increase the frequency of micronuclei.

(*b*) Strain α 2 (Fig. 33)

This is a "part-mutation" beardless speltoid strain from the variety Iron (*T. vulgare* var. *lutescens*). Two beardless speltoid and two beardless het speltoid plants were obtained from Prof. H. Nilsson-Ehle and mature progeny were obtained from seed sown in England in March 1930. None of their progeny headed when sown in Canada the following spring. The two speltoids bred true. The two het speltoids gave 17 N, 25 H, 22 Sp, and 1 normal-het speltoid chimera, all beardless. The deviation from a 1 : 2 : 1 ratio though considerable is not significant ($\chi^2 = 3.84$; $P = .10 - .20$).

Two normal plants regularly had 21_{II}; in only one of the large number of nuclei examined were two chromosomes unpaired. Four het speltoids all had 21_{II} including one that sometimes appeared to be very slightly heteromorphic (Fig. 22) and failure of association of one pair was noted in a few cells. Three speltoid segregates all had 21_{II}.

The chimera had one het speltoid and two normal heads. By a fortunate chance, one culm had been fixed—of course before the phenotype was determinable. It had one heteromorphic bivalent (Fig. 41), an arm of one chromosome having been lost. Genetic analysis would, of course, be necessary to prove that it is this deficiency which has given rise to the het speltoid phenotype. When the plants produced from seeds of this chimera failed to head in 1931 they were transplanted into the greenhouse but only one

FIG. 22. *α 2 het speltoid with 20_{II} plus one possibly heteromorphic bivalent—second from right.*

survived to head the following July and it was a normal. In 1932 vernalization of the remaining seeds was attempted, but the treatment given failed to cause heading and none of the plants survived the following winter in the greenhouse. It was noted that progeny of the het speltoid head were weaker than those from the normal head. The coincident appearance of the het speltoid spike and the occurrence of a deficiency similar to that of some γ het speltoids, together with the reduced vigor of the progeny, render it probable that we have here direct evidence on the origin of one het speltoid—through segmental chromosome deficiency occurring in somatic tissue; it is most unfortunate that the genetic evidence needed to complete the case was unobtainable.

IV. Interpretation of the Segregation Ratios

A. General Genetic Data

Nilsson-Ehle (24) showed that while the deviations of Series A ratios from a simple 1 : 2 : 1 could readily be accounted for by "differential gametic elimination", some more comp'ex explanation was necessary for Series B and C ratios. He applied the concept of heterogamy (then current as a corollary of the "Reduplication" theory of linkage) and contrived a formal explanation which he proposed to test experimentally. In 1927, in his paper on part-mutations (25), he mentioned that both his own and Lindhard's data had shown such an explanation to be inadequate. By that time, of course, the reduplication theory itself was discarded and the chromosome theory of linkage thoroughly established. Winge's chromosome studies of speltoids had appeared and Nilsson-Ehle recognized that they shed new light on the problem but he also pointed out serious limitations in Winge's hypothesis.

Lindhard (16) considered that differential zygotic elimination also was involved in determining the ratios. Later (18) he attempted also an explanation involving a pair of factors L and l which were assumed to influence both the compactness of the spike and the segregation ratios.

Leaving aside for the moment the cytological data now available, the facts which must be considered in interpreting the ratios are as follows:

Series A or α ratios deviate from the 1 : 2 : 1, expected from random segregation and recombination of a unitary difference, only in having an increased proportion of normals, usually at the expense of homozygous mutant progeny. Either (*a*) "certation", i.e., reduced functioning of mutant gametes when in competition with normal ones, or (*b*) differential zygotic elimination, i.e., lowered viability of het speltoid and even more so of speltoid zygotes, would suffice to account for the deviations.

Series B or β ratios must depend upon some mechanism which assures the production of a large preponderance of mutant ovules while mutant pollen must either be almost entirely lacking, or else mostly nonfunctional. The converse possibility of functional mutant pollen and nonfunctional mutant ovules (which would, of course, fit self-fertilized het speltoid ratios) is ruled out by the results of reciprocal crosses: het speltoid ♀ × normal ♂ gives ratios similar to self-fertilization of het speltoids while the cross normal ♀ × het speltoid ♂ gives almost entirely normal offspring—see later.

Series C ratios as defined by Nilsson-Ehle comprise slightly more normal than het speltoid progeny while speltoid segregates are rare. Evidently, Series C mutant pollen functions less often than in typical Series α, while the excess of normals demands either, (*a*) that mutant ovules function, and probably are formed, less often than in Series α, or else, (*b*) that there is a greater zygotic elimination of het speltoids in Series C. In the strains we classify as Series γ there is no excess of normal over het speltoid segregates. There is therefore in them no need to assume the production or functioning of more normal than mutant ovules as Nilsson-Ehle did for Series C. It seems possible that lowered viability of het speltoid zygotes during the winter, together with chance variation, may be responsible for the excess of normals in some of Nilsson-Ehle's strains. Until this is ruled out it is unnecessary to speculate further, though it is theoretically possible that het speltoids could sometimes arise through trisomy involving "primary" speltoid genes. If such occur, they could readily produce more normal than mutant ovules, as do some subcompactoids. There is no clear dividing line between γ and α ratios and the only definite genetic reason for differentiating them is that Series β het speltoids continuously arise from γ and scarcely ever from typical α. Their continuous, though irregular, occurrence indicates that some abnormal segregation process occurs during ovule formation in γ het speltoids.

B. General Cytological Data

The cytological studies add the following data:

In Series α the normal chromosome number is present in all three classes of segregates and there is little evidence of any grossly abnormal chromosome behavior during meiosis. The alternative explanations that have to be considered to account for the origin and segregation ratios of α het speltoids are (*a*) gene mutation and (*b*) chromosome aberration, most probably of the deficiency type.

In Series β the presence of an unpaired C chromosome, one or both chromatids of which very frequently fail to become included in the spore nuclei, provides the mechanism for the production of a great excess of mutant (20-chromosome) gametes in both pollen and ovules.

In Series γ there is in one member of a pair of heteromorphic chromosomes a deficiency that both affects the regularity of their meiotic metaphase association and permits the expression of the speltoid characters. When these heteromorphic chromosomes (CCd in some strains and CCts in others) are associated and disjoin normally, mutant and normal gametes are formed in equal numbers. When they are unassociated, both behave as univalents and, as in Series β, some or all of the chromatids may be left out of the spore nuclei. Gametes with only 20 chromosomes are therefore formed fairly frequently and an ovule possessing neither C nor Cd or Cts when fertilized by normal pollen produces a Series β het speltoid.

C. Ovule Competition and Embryonic Viability

While the above features establish the major differences between α, β, and γ Series, we have to consider further various factors which may modify the ratios characteristic of each. In all three Series, reciprocal crosses show that mutant pollen is at a disadvantage when in competition with normal, the degree increasing in the order α, γ, β (see section F, Table XII).

The question remains, are mutant ovules at any disadvantage? Kihara (14) attempted to explain the segregation of pentaploid wheat hybrids in part by the assumption of either ovule sterility, or zygotic elimination during the embryonic stage and his students up to and including Nishiyama (28) and Uchikawa (36) have continued to maintain this for both fatuoids and speltoids, as well as interspecific hybrids. Watkins (38, 39), however, showed clearly that under the conditions at Cambridge ovules with chromosome numbers ranging from 14 to 21 almost all function and produce viable seed. In view of this, it would be surprising if mutant ovules, or embryos, of any of the speltoid Series were seriously handicapped, except perhaps under very adverse conditions and strong competition. Lindhard (16) found in Denmark that het speltoids of Series β were only 64% fertile, while their normal sibs were 84% fertile and he used these data in his interpretation of the ratios, assuming that het speltoid embryos were less, and speltoid embryos much less, viable than normals. Uchikawa (36) has reported that in Japan Series α het speltoids are only about 1% less fertile than their normal sibs, but that fertility in Series γ is 6.5% and in Series β 12.56% lower. On the other hand, Nilsson-Ehle (24) found in Sweden no appreciable difference: In Series γ the percentages of sterile flowers were 6.3 and 7.4 and in Series β they were 9.7 and 9.4 in normals and het speltoids respectively.

These apparently contradictory data can actually be harmonized without taking into account possible differences in the growing conditions. First,

Nilsson-Ehle (24) counted seed production only in the first two flowers of each spikelet, whereas Lindhard counted all florets. Watkins (38) pointed out that: "Whether or not a grain is set in the third, fourth, fifth, or higher flowers of a spikelet depends more on the vigour of the tiller bearing the ear in question than on the amount of sterility; in small ears it is rare to find flowers above the third giving a grain while in ears that are well developed even the sixth flower may sometimes do so. Mean number of grains per spikelet is therefore too much influenced by conditions other than amount of sterility to be a measure of this latter quantity." It was found that the total seed production per open-pollinated spike is similar in normals and het speltoids of both Series β and γ when grown under favorable conditions in England (Table X). In counts restricted to the most favorably situated florets, that is, to the first two florets of spikelets situated between the basal spikelet and the apical three, there was, surprisingly, a smaller actual proportion of sterile florets in the het speltoids than in their normal sibs (Table XI), but in no case was the difference statistically significant. A third test was made by removing, soon after heading, the basal and three apical spikelets completely and, from the remaining spikelets, all florets above the first two, which is the procedure usually followed when crosses are to be made. These heads were left uncovered and free to effect self-fertilization, or to be cross-fertilized occasionally, as in the controls. The progeny ratios obtained from their seeds were in no case different from seeds of untreated heads. In Strain β 1 the seeds from treated heads gave 9 N : 39 H : 2 Sp while the control gave 8 N : 42 Het : 0 Sp. In Strain β 2 the ratios were 12 : 35 : 0 and 19 : 74 : 0. In Strain γ 1 they were 31 : 42 : 5 and 34 : 39 : 2 and in γ 4 they were 41 : 45 : 1 and 37 : 48 : 0. The average seed weight from treated and untreated heads was also similar. There were, however, more cross-fertilized seeds on the treated heads; their progenies were excluded from the above ratios.

TABLE X

THE NUMBER OF SEEDS PER SPIKE IN NORMAL AND HET SPELTOID SIBS*

Strain	Normal			Het speltoid			Difference in number of seeds
	No. of plants	No. of spikelets per head	Mean number of seeds	No. of plants	No. of spikelets per head	Mean number of seeds	
β 1	13	20.8 ± 0.2	37.8 ± 2.6	13	20.2 ± 0.3	42.9 ± 1.8	5.1 ± 3.1
β 3	22	25.3 ± 0.3	—	50	24.4 ± 0.2	—	—
β 4	17	21.2 ± 0.3	35.7 ± 2.2	17	21.7 ± 0.3	38.2 ± 2.6	2.5 ± 3.4
γ 1	25	19.9 ± 0.4	39.2 ± 2.5	22	19.5 ± 0.4	38.6 ± 2.0	0.6 ± 3.1
γ 4	18	21.7 ± 0.3	40.5 ± 2.1	18	20.9 ± 0.3	44.3 ± 2.6	3.8 ± 3.3

* *Counted on best spike from each plant. Plants grown under very favorable conditions at John Innes Institution, Merton, in 1929.*

TABLE XI

STERILITY OF MOST FAVORABLY SITUATED FLORETS IN NORMAL AND HET SPELTOID SIBS*

Strain	Normal		Het speltoid		Significance of difference
	No. of plants	Percentage of sterile 1st or 2nd florets	No. of plants	Percentage of sterile 1st or 2nd florets	
β 1	13	8.23 ± 2.67	13	4.04 ± 2.56	4.19 ± 3.68
β 3	22	25.68 ± 3.05	50	16.09 ± 2.15	9.59 ± 3.73
β 4	17	9.61 ± 3.14	17	6.76 ± 2.35	2.85 ± 3.92
γ 1	25	10.26 ± 3.68	22	4.32 ± 1.26	5.94 ± 3.88
γ 4	18	6.21 ± 1.80	18	2.14 ± 2.30	4.07 ± 2.91

* *Calculated from counts made on one side of best spike from each plant.*

Uchikawa's (36) finding of appreciably greater sterility in γ and still more in β het speltoids than in their normal sibs demands further consideration for he determined the percentage of seed setting only in the first and second florets of each spikelet. The clue to the discrepancy between his results and those of Nilsson-Ehle, Watkins, and ourselves seems to lie in the fact that before flowering time he covered with parchment bags the spikes which were to be used for the later examination of mature grains. This is a very necessary procedure if all natural crossing is to be avoided, but it introduces a complication which he failed to take into account. Watkins (38) found 13% fewer seeds set on bagged, but otherwise untreated, F_1 pentaploid hybrid heads and 23% fewer on heads with top and bottom spikelets and all upper florets removed, than on untouched heads allowed to self-fertilize naturally. He concluded that where the amount of germinable pollen is small, as in these hybrids, "a comparatively slight change in external conditions may turn the scale from failure to success". The same reasoning, *mutatis mutandis*, will account for Uchikawa's results, including the greater sterility in β than in γ het speltoids under similar conditions: in both it is chiefly normal pollen that functions and in γ het speltoids approximately half the pollen is normal, while in β het speltoids not more than one-fifth of it has the normal chromosome constitution.

It is thus not established that het speltoids show a greater degree of sterility than their normal sibs and there is, therefore, no indication that mutant ovules are at a disadvantage, or that het speltoid, or speltoid embryos, are less likely to begin development than are normal ones. There remains the possibility that they may develop less satisfactorily and that seeds carrying het speltoid, or speltoid embryos, may therefore have poorer germination or poorer seedling growth.

Uchikawa's finding of 50 and 55% fertile seeds on β and γ speltoids is surprising, especially in the former. Our β speltoids are sterile unless they have one or more non-C chromosomes in excess that offset the absence of both C chromosomes (see β 3), and Sears (32) reports that his 40-chromosome

speltoids (nullisomic IX) are male sterile. Further (on the basis of assumptions with which, incidentally, we disagree) Uchikawa's own calculations (36, p. 56-7) on β ratios imply that only about 6% of the speltoid embryos formed by het speltoids develop into fully formed grains; it would therefore be surprising if similar embryos formed on speltoids, which are typically weak plants, should be so much more viable. Further, if Uchikawa's β speltoids were even only partially male sterile, their progeny by open pollination would be expected to comprise many het speltoids. He does not present progeny tests, so that one cannot check this, but lacking evidence to the contrary it is reasonable to assume either (*a*) that the embryos he counted were predominantly het speltoids (resulting from natural crossing) or (*b*) that he was dealing, not with typical 40-chromosome β speltoids, but with some of those commonly occurring types that have extra non-C chromosomes.

D. Later Zygotic Elimination

Several workers with cereals have assumed differential zygotic elimination some time between germination and maturity to account in part for aberrant progeny ratios and particularly for variations between the ratios obtained from apparently similar heterozygotes in different seasons. Lindhard (16) maintained that such zygotic elimination may have been responsible for his obtaining in 1916 only three or four times as many het speltoids as normals in his β strain that characteristically gave 1 N : 8 Het. His strain was fall-sown and in some seasons as few as 10% of the plants survived the winter. In 1918, 81.25% of the seeds from normal segregates produced mature progeny, compared with 66.3% of those from sib het speltoids. From these data, he assumes a 1 : 5 ratio should really be 1 : 7 and so on. Unfortunately, he does not present in his tables the number of seeds sown and germinated, but only the numbers of plants of the various types harvested. It is therefore impossible to check his hypothesis. Further, many of the ratios he considers distinct are really not heterogeneous by modern χ^2 tests. Detailed germination results are not given by Philiptschenko (29), but he recorded that only 32% of the progeny of het speltoids survived and that only 28% were fully developed plants, while there was 45% survival in the progeny of normal sibs. From this and the ratio he calculated that het speltoids have only 70-80% and speltoids only 8.5% the viability of normals, and attempted to explain the deviation of his γ ratio from 1 : 2 : 1 on the basis of differential mortality of the three classes of segregates.

The only tabulated germination data seem to be those of Uchikawa (36) who unfortunately combines four years' results, as he also combines β segregation ratios, though he presents annual ratios for Series α and γ. His results will be considered in conjunction with our own germination data and the finding of Åkerman (1) that the weights of 1000 seeds from normals, het speltoids, and speltoids of a strain classed as Series A (but which we should call γ) were 41.4 ± 0.31, 36.0 ± 0.30, and 30.5 ± 0.42 gm. respectively. These

weight differences might easily be correlated with differential germination, or they might determine differential elimination of seedlings before emergence above ground.

We have found that the percentage of emergence above ground of seeds from normals is usually greater than that of seeds from het speltoids. However, statistical analysis of β emergence data showed that sowings from normals were relatively uniform, while those from β het speltoids had highly significant heterogeneity mainly associated with seasonal differences, though there are also significant but lesser differences between strains and between different progenies of one strain within seasons. It seems probable that the great heterogeneity of the tests on seeds from het speltoids is due to the low germination percentage of some progenies of plants which bore few seeds. Since larger progenies are necessary to test the ratio type of a het speltoid than to determine the homozygosity of a phenotypic normal, there is always the probability that the sample of seed picked from the former will comprise some smaller or more shrunken seeds, particularly if some of the plants are not prolific. Uchikawa (36) showed lower percentage germination for seeds from het speltoids than normals in each of his four β strains but the difference was not significant in any one strain. In any event, his percentages, being based on four years' lumped data, are conclusive only if their components were not heterogeneous.

Uchikawa's sowings of seeds from β or γ speltoids showed an appreciably lower percentage emergence than those from het speltoids or normals, from which he concludes there is differential elimination in early stages of speltoid zygotes and that this helps to explain the β and γ ratios. However, his difference in emergence is based on much smaller sowings of seeds from speltoids than from het speltoids or normals, and as these smaller sowings are not in rounded or regular numbers, the probability arises that, as in our own het speltoid sowings, there may have been included an undue proportion of small or shrunken seeds due to the necessity of using all, or almost all, seeds from the characteristically poorly developed speltoid plants. Further, no evidence is presented that the seeds from β or γ speltoids were themselves of the β or γ speltoid constitution. In any case, in his argument from these data that differential elimination of speltoid zygotes borne on het speltoids helps to explain the β and γ ratios, Uchikawa fails to distinguish between a direct effect of genetic constitution on the viability of the zygote and the possibility that the constitution of the parental plant affects the viability of any, or all, seeds borne by it. It is unlikely that speltoid seeds borne by poorly developed speltoid plants are as viable as speltoid seeds produced by vigorous het speltoids, yet some of his calculations could be taken to indicate that they are more viable.

Lindhard (16) thought that the weights of seeds borne by het speltoids were correlated with their genetic constitution. His data are very striking but not consistent with any simple correlation or hypothesis. We have weighed and

sown separately the largest, smallest, and medium grains of two β, two γ, and six α-γ het speltoids. The test involved 468 seeds and no correlation was found between seed weight and segregation ratios.

Differential survival of the three classes of segregates in the field was postulated by Lindhard, Philiptschenko, and Uchikawa. Involved in their reasoning is the fact that β and γ (or C) speltoids are generally smaller and weaker plants than their normal and het speltoid sibs. But in all cultures recorded except Lindhard's, het speltoids are vigorous plants, taller than and having as many, or almost as many, culms as their normal sibs. Lindhard (16) used plant weights as an additional measure of vigor. In the first year of his experiments, 1918, he found the average weight of normals and het speltoids to be 63 and 36 gm. However, in later years, his results were very variable. For example, in one family comprising 33 normals and 247 het speltoids, grown in 1921, the respective weights per plant were 25 and 52 gm., while in one related family, also of Series β, they were 77 and 48. In a related γ family they weighed 60 and 46 gm. while in an α strain the normals, het speltoids, and speltoids averaged 57, 84, and 7 gm. each. Such a disproportionately small size of the speltoids is atypical for Series α and more like that for β, or γ. Lindhard remarked on the discontinuous variation in weights that occurs throughout his sublines of speltoids. On quite other bases it has been shown (12) that there is strong reason to suspect that some of Lindhard's "speltoids" were really hybrids with *T. spelta*, or its derivatives. That assumption might well explain these weight variations, as well as many other puzzling features by which Lindhard's results differ from those of other workers. Some varieties of *T. spelta* have more slender straw and weigh less than most varieties of *T. vulgare* and we know from speltoid data that there are size and vigor genes in the C chromosome of the latter. Crossing over between C and its homoeologue from *T. spelta* would suffice to explain the more random association of weight with other characteristics in later generations.

The heights and numbers of culms have been recorded for practically all plants in our cultures and many have been weighed. In none of our strains of any of the three Series are het speltoids shorter or of smaller average weight than their normal sibs when comparisons are made within one season. In Series β and γ, speltoid plants are characteristically smaller and weaker than their normal and het speltoid sibs. It would not therefore be surprising if under adverse growing conditions they should be differentially eliminated. This might apply especially to fall-sown strains such as Lindhard's and some of Åkerman's, and Nilsson-Ehle's. Following late sowing and a very severe winter Åkerman (1) found 12.8% survival from normals and only 3.5% from their γ speltoid sibs. Following two mild winters, the survival rate was 55 to 57% from sowings of normals and speltoids alike. It is usually higher for spring sowings. Though Uchikawa (36) postulates differential elimination of speltoids to explain β and γ ratios, his Tables 11 and 12 show

almost perfect survival from crosses between het speltoids and speltoids which give a high proportion of speltoid progeny. He does not give survival rates for the progeny of het speltoids.

Our progenies of β het speltoids are heterogeneous, by statistical test, for survival to maturity as well as for emergence. This heterogeneity is not, however, reflected in heterogeneity of segregation ratios within or between seasons or strains.

The factors which cause the fluctuations in emergence and survival cannot be shown to act differentially on the three classes of segregates in our cultures. The tests do not rule out the possibility that there has been a slight and fairly constant greater elimination of speltoid segregates, but, as will be shown, this cannot be an important factor in determining segregation ratios or the differences between the three Series, since it is overshadowed by the factors of gamete formation and functioning. In the absence of proof of differential zygotic elimination, the ratios determined at plant maturity under any ordinary conditions of growth give the best available estimate of the frequencies with which the three classes of zygotes are formed by het speltoids.

E. Spore and Gamete Formation

(a) Series α and β

In Series α, it may be assumed that mutant and normal gametes are formed in equal numbers. In Series β, an excess of mutant gametes, which function on the female side, must be postulated. An estimate of the proportions of 20- and 21-chromosome pollen formed by β het speltoids is readily obtained from counts of micronuclei in the pollen tetrads. During the first meiotic anaphase, the component chromatids of the univalent C chromosome characteristically separate and pass towards opposite poles. They may reach the poles in time to be included in the dyad nuclei, or either one, or both, may be left out on the spindle. During the second meiotic division, these "daughter univalents" do not ordinarily divide again. If they have been included in the dyad nuclei, they may go to either pole of the second division spindle. If they have been left out in the dyad cytoplasm, they can, apparently, be picked up during the second division. In either case, they may either be left out in the cytoplasm, or included in the tetrad nuclei at the end of the second meiotic division. If left out, they apparently take no further part in gamete formation, but form micronuclei. The presence of two, one, or no micronuclei in a pollen tetrad therefore indicates that it will form four, three, or two gametes, respectively, with only 20 chromosomes each. Deviations from this expectation will, of course, occur if chromosomes other than the univalent C fail to pair and segregate normally. We have found this to occur in varying frequencies up to as high as 6% of the pollen mother cells. The calculated proportion of 20-chromosome, speltoid-producing gametes will therefore be an overestimate.

From one het speltoid of strain $\beta 1$, two of $\beta 4$, and one of $\beta 5$, samples of 150 pollen tetrads each were examined and 96 tetrads from a β het speltoid that

arose from a subcompactoid. The proportions of 20- : 21- chromosome male gametes they would be expected to produce were 84 : 516, 99 : 501, 65 : 535, 82 : 518, and 30 : 354, i.e., 14.0, 16.5, 10.8, 13.7, and 7.9%, respectively of the pollen would be normal. Uchikawa (36) from counts of 604 tetrads calculated that 18.9% of the pollen is normal in his β het speltoids. He implies that variability was encountered, see below, but gives no data from which its extent can be determined.

In view of the low frequency with which 20-chromosome pollen usually functions when in competition with an abundant supply of normal pollen (see next section), the determination of the frequency with which it is formed is of less direct significance than it is of indirect value in giving some indication of the relative proportions in which mutant and normal ovules are formed. As shown above, both types of ovules must be functional since there are no more sterile florets on het speltoid than on normal spikes. It is, unfortunately, too laborious an undertaking, especially in view of the probable variability, to determine directly on a statistically significant scale the proportions of mutant and normal ovules. Watkins (38, 39) found that in pentaploid hybrids there was no evidence of any appreciable difference in the frequency with which univalents are lost during androsporogenesis and gynosporogenesis. He pointed out that while chromosomes left out of the dyad nuclei and picked up by the transverse second division spindle of androsporogenesis have a chance of getting into a functional pollen grain, the same does not hold during ovule formation. In gynosporogenesis the two spindles have the same orientation and chromosomes left out of the dyad nuclei are therefore not likely to be carried into the innermost, potentially functional, gynospore. There may therefore for this reason be a somewhat greater proportion of 20-chromosome ovules formed than of pollen grains, but as Watkins pointed out for his hybrids that had seven univalents, the difference is hardly likely to be significant in affecting progeny ratios that may be influenced by so many other factors. This should be even truer for β het speltoids that have only one univalent, and since we do not know what other factors may influence its inclusion or loss, we can only proceed on the assumption that it is similar in gynosporogenesis to that determined from pollen tetrad counts and see how closely this assumption fits the genetic data.

Quite unexplained is Uchikawa's report that a 41-chromosome speltoid of the constitution 40 + C^{α} gave 42-, 41-, and 40-chromosome speltoids in the ratio 1 : 1 : 0.1. Unless natural crossing or some other chromosome aberration is involved, this ratio would indicate that the mutated univalent, C^{α}, is included or left out of the gynospore nuclei with equal frequency, whereas a normal C chromosome when univalent in a 41-chromosome het speltoid gets left out 3 to 5 times as often as it gets included.

(*b*) *Series* γ

There are more indeterminables in γ than in β het speltoids. When the normal C chromosome and the C lacking a segment, "Cd" or "Cts", are

associated at metaphase they presumably segregate normally giving equal numbers of normal and mutant spores. When unassociated at first meiotic metaphase they both behave like the univalent C of β het speltoids and from none to all four of the "daughter univalents" may therefore get into, or be left out of, any of the tetrad nuclei. When there is a micronucleus in each quadrant of the tetrad, there are obviously (apart from the possibility of chromosomes other than C being left out) four spores with 20 chromosomes each. If there are three, then three spores have 20 and one has 21. When there are only two micronuclei, their relative position has to be considered. If they lie in diagonally opposite quadrants, there are two spores with 20 and two with 21. If they are in adjacent quadrants, two "daughter univalents" may have been included, one in each, or both in one of the other quadrants. Observations were, when possible, made in the brief stage after formation of the nuclear membranes in the tetrad and before the dyad arrangement is obscured by the development of the secondary, transverse walls. If observations are made after the tetrad walls are fully formed, the assumption may be made, for purposes of calculation, that micronuclei in adjacent quadrants had previously been in the same or different dyads with equal frequency. When there are no micronuclei, all quadrants may have 21 chromosomes, as expected if C and Cd or Cts have been paired, or, if they were unpaired, some gametes may have 22 and others only 20 chromosomes.

In het speltoids of strain γ 2 the C and Cd chromosomes were unpaired in 13.6% of 154 first metaphase pollen mother cells. In 372 tetrads only 9.4% had micronuclei. Assuming these to be representative samples and all micronuclei to be constituted of C or Cd chromosomes, 4.2% of the normal tetrads resulted from pollen mother cells with C and Cd unpaired and their nuclei with 20, 21, and 22 chromosomes comprised 1, 2, and 1% respectively of the total sample of spores. From this, strain γ 2 het speltoids are calculated to produce 20-, 21-, and 22-chromosome pollen in the proportion of 6.1 : 92.4 : 1.5. Details of the method of calculation were given by Smith (33, 34). It may be assumed that the 92.4% of 21-chromosome pollen comprises equal numbers with 20 + C and 20 + Cd, and similarly the 1.5% comprises 21 + C and 21 + Cd. The foregoing all assumes, of course, that C and Cd univalents have similar chances of inclusion.

With so many possible variables, the only important conclusion to be drawn on Series γ is that 20-chromosome pollen is formed sufficiently often to require consideration in the interpretation of the ratios. Used as an indication of the frequency with which 20-chromosome ovules are formed, it gives an estimate of the frequency with which β het speltoids may be expected to arise from γ het speltoids and this can be compared with the observed frequency in various strains.

F. Certation

Certation, the measure of competition between diverse pollen grains in effecting fertilization, is evidently of great significance in determining the

β segregation ratios. Crosses of normals or speltoids with het speltoids provide a measure of the frequency with which the mutant and normal pollen grains function, but not, of course, directly a measure of their relative innate capacity to function, since we have first to take into account the frequency of formation and secondly to recognize that the total amount of pollen available, as well as many other environmental factors, may influence the effective competition.

(*a*) Series α and γ

No definite estimate can be made of the degree of certation in Series α for the reason that α grades into γ. By definition, "good" α strains give ratios approaching 1 : 2 : 1 though almost invariably with some shortage in the numbers of het speltoids and especially of speltoids. Certation can therefore be occurring to only a limited extent in them. The theoretical limit of certation would be reached in γ het speltoids that give 1 : 1 : 0 ratios. Some strains closely approach this limit.

Nilsson-Ehle (24) showed that in a γ strain which gave 135 normals, 138 het speltoids, and no speltoids on selfing, the cross of het speltoid by normal gave nine normals and seven het speltoids, while the reciprocal cross gave 29 normals only. When in later generations a very weak speltoid segregate was obtained, Nilsson-Ehle (23) related the degree of weakness of the homozygous mutants to the degree of incapacity of the mutant pollen to effect fertilization. In a strain intermediate in our classification between α and γ, he obtained five normals and five het speltoids from the cross het speltoid by normal and 10 normals to one het speltoid from the reciprocal cross. Åkerman (1) obtained 126 normals and 22 het speltoids from crossing normals with het speltoids. Sib het speltoids on selfing gave 45 normals, 53 het speltoids, and three speltoids. He therefore classified this as Series A, while we call it γ. In any case, it is of the chromosome type that *forms* mutant and normal gametes in approximately equal proportions. The ratio obtained from the cross therefore indicates that normal pollen in this case had almost a 6 : 1 advantage over mutant pollen. Crosses of normals by our $\gamma 4$ het speltoids gave 72 normals and three het speltoids (Table XII). In this case, normal pollen had a 24 : 1 advantage. Uchikawa's (36) crosses of normals by het speltoids derived from our $\gamma 4$ strain gave 76 normals and eight het speltoids, while his crosses of speltoids by their het speltoids gave 52 het speltoids and six speltoids. Together, these crosses show the normal pollen having a 9 : 1 advantage.

(*b*) *Series* β

Crosses of speltoids by β het speltoids were first made by Nilsson-Ehle (24) who obtained nine het speltoids and no speltoids, from which he concluded that the normal pollen of β het speltoids has a very great competitive advantage and that this is very important in determining β ratios. This was confirmed by Lindhard (16) who from crosses of normals by β het speltoids got 103 normal and no het speltoid progeny. From the same cross in strain $\beta 4$, we got 91 normals and no het speltoids.

TABLE XII

RECIPROCAL CROSSES

Female parent	Male parent	Seeds sown	Seeds germ'd	Percentage progeny			Total matured	Percentage gross survival
				N	Het	Sp		
β 4 het	N	108	87	25.9	74.1	0.0	85	78.7
β 4 het	Selfed	275	230	21.2	78.8	0.0	231*	83.6
β 4 N	β het	100	91	100.0	0.0	0.0	90	90.0
γ 1 het	N	21	20	50.0	50.0	0.0	20	95.2
γ 1 het	Selfed	192	167	42.2	52.6	5.2	154	80.2
γ 4 het	N	75	51	55.3	44.7	0.0	47	62.7
γ 4 het	Selfed	252	207	47.3	52.2	0.5	205	81.3
γ 4 N	γ het	86	75	96.0	4.0	0.0	75	87.2

* *Including one dwarf, unclassifiable plant.*
All the above plants were grown in 1929.

Uchikawa (36) on the other hand, found a much smaller degree of certation. From crosses of normals by β het speltoids derived from our β4 strain, he got 92 normals and 14 het speltoids, which indicates that mutant pollen effected 13.2% of the fertilizations. From crosses of α, β, and γ speltoids by β het speltoids he got 38 het speltoids and seven speltoids, indicating 15.6% fertilization by mutant pollen. These are higher rates of functioning than in his own self-fertilized β het speltoids, as judged from their proportion of speltoid segregates. Uchikawa believes that in selfed β het speltoid the mutant pollen actually functions as well as in his crosses, but that about 94% of the resultant β speltoid progeny are eliminated. We shall consider two other possibilities:

(1) That Uchikawa used small amounts of pollen in his crosses, relative to that available in selfings, and thus reduced competition for the mutant gametes.

(2) That a change has occurred in his strain analogous to though not as extreme in its genetic effect as that which has a number of times produced "modified β" from het fatuoids (27, 31, see also 5). Uchikawa recorded that in 18,357 p.m.c. of β het speltoids there were $20_{II} + 1_I$ and in only 122 were there any other abnormalities. In modified β het fatuoids there are $20_{II} + 1_I$ as in β het fatuoids or β het speltoids and we have so far been able to find no definite cytological difference. But whereas in β het fatuoids the mutant, 20-chromosome, pollen is very largely nonfunctional, it is highly functional in modified β het fatuoids.

Uchikawa's β speltoid segregates also differ from those of most other workers in being partially fertile instead of almost completely sterile. The "nulli IX", 40-chromosome β speltoids that Sears (32) has obtained in his current analysis are as sterile as ours. He reports (personal communication) "I have grown several dozen nulli-IX plants over a period of years, and to my knowledge not a seed has set on any one of them from self-pollination, in spite of reasonably

good female fertility. As a matter of fact, indehiscence of anthers is the rule, although I cannot say definitely that an occasional anther does not dehisce."

All of the vigorous, more or less fertile, speltoids arising sporadically from Series β which we have examined have chromosomes other than C in excess numbers. Sears (32) has shown that normal pollen has no competitive advantage over pollen lacking chromosome II when it has chromosome XX in excess. This compensation phenomenon applies in varying extent to other chromosomes, from which it follows, as Sears points out, that there will be a selection in favor of compensating combinations. Since Uchikawa found only 40 chromosomes in an extraordinary number of p.m.c. it cannot be whole chromosome compensations that are involved in his β speltoids, but it could be a translocated segment.

G. Correlation of Genetic with Cytological Data

Attempts to compare observed genetic ratios with theoretical expectations based on chromosome loss in pollen tetrads have been made by Smith (34) and by Uchikawa (36). Further analyses have now been made of Series β and of α and γ jointly.

(*a*) *Series* β

Smith's original estimate (34) of the frequency with which 20-chromosome pollen functions was based on the frequency of speltoids found in the progeny of selfed het speltoids. It was concluded, from the fact that the expectation based on this and the tetrad counts was very close to the ratio observed, that it is unnecessary to assume zygotic elimination of speltoids. The speltoids, however, comprise only about 1% of the progeny, which makes it unwise to base expectation on them. Further, statistical tests have since shown that the tetrad counts are heterogeneous.

Uchikawa's (36) argument, in brief, is: Pollen tetrad counts show that 21- and 20-chromosome pollen grains are produced in the ratio of 1 : 4. Crosses of het speltoids by normals show that the ovule proportion is the same. The cross of normal by β het speltoid gave 15 normals and three het speltoids; thus 21- and 20-chromosome pollen is functioning in the ratio 1 : 0.2. Hence, on selfing β het speltoids a ratio of 1 N : 4.2 Het : 0.8 Sp is expected. The observed ratio was 1 : 4.5 : 0.05 and the discrepancy is accounted for by the assumption that 15/16 of the speltoid offspring (12.5% of the total zygotes) are eliminated before reaching maturity. His general conclusion is that the ratios are due: (*a*) to a 4 : 1 ratio of 20- and 21-chromosome gametes, (*b*) to certation favoring normal gametes in the proportion of 20 : 1, and (*c*) zygotic elimination of speltoids. The fact (*a*) that the ratios vary considerably and (*b*) that according to his own statement "frequency of chromosome elimination is almost necessarily affected by various environmental conditions" does not appear to have been considered sufficiently in reaching these conclusions.

Variation in the proportion of normal and mutant ovules is apparently the only factor that could appreciably affect the ratio of normals to het speltoids. For instance, if one accepts Uchikawa's figure of 1 : 4 for ovules, but changes the assumed ratio of functioning normal and mutant pollen from 1 : 0.2 to 1 : 0.02, the expected zygotic ratio would be changed only from 1 : 4.2 : 0.8 to 1 : 4.02 : 0.08. If instead of 1 : 0.2 functioning one assumes 1 : 0.0125, the zygotic ratio expected would be 1 : 4.0125 : 0.05. In other words, if the ovule formation ratio remains the same, a change in the assumed frequency of functioning of mutant pollen from 1/5 to 1/80 of that of normal pollen would change the expected ratio of normals: het speltoids only from 1 : 4.2 to 1 : 4.0125. This latter change is insignificant when compared to the variations in N : H ratios that have to be accounted for. On the other hand, the change in the assumed frequency of functioning mutant pollen would reduce the expectancy for the speltoid class by 15/16.

The assumption of great differential elimination of speltoid segregates, which is an integral part of Uchikawa's conclusions, seems unnecessary on the basis of his own, Sears', or our data. Sears (32) found 1.5% speltoids in a population of 134 plants from 135 seeds. Certainly zygotic elimination after the stage of seed formation was not a significant factor in this case. On the other hand, variations in certation especially when chromosome deficiencies or aberrations are involved, are well known to be affected by differences in the amount of pollen applied and by the nature of the deficiency or other aberration in the chromosome primarily concerned. We conclude therefore that the frequency of speltoids obtained from β het speltoids depends: (*a*) largely on the degree of certation between 20- and 21-chromosome pollen, (*b*) to a much lesser degree on zygotic elimination, and (*c*) very little on the proportion of 20- : 21-chromosome pollen formed, since there is always a great excess of the former.

The primary characteristic of β ratios, the great excess of het speltoids (1 : 3, or usually more, instead of the 1 : 2 on Mendelian expectation) depends jointly on certation and the fact that female gametes with 20 chromosomes are formed three or more times as often as normal ones with 21 chromosomes and are functional. Sears found the female transmission rate of nullisomics to be about 75% regardless of the chromosome concerned. In β het speltoids it is commonly somewhat higher than this. The fluctuations in the proportion of normals and het speltoids derived from self-fertilized het speltoids are predominantly due to the frequency with which the univalent C chromosome is left out of the gynospore during meiosis.

As for the use of pollen tetrad data in calculating the probable proportion of 20- and 21-chromosome gynospores, it has to be said that although the ratios are thereby reasonably well fitted there are some ratios that indicate a higher proportion of normal ovules than are estimated from any of our, or Uchikawa's, tetrad counts. This may mean that the C univalent is actually lost less often in gynosporogenesis. The discrepancy could, however, be

explained either as resulting from the variability of the tetrad counts, or to their giving an overestimate of C-chromosome loss because other chromosomes may have formed an appreciable proportion of the micronuclei counted.

(*b*) *Series α and γ*

From crosses of Series A (our γ) het speltoids by normals, Åkerman (1) obtained 25 normals and 25 het speltoids. From similar crosses in strains γ1 and γ4 we got 26 and 21. Uchikawa (36) got 9 : 7, 17 : 15, and 16 : 14 in three crosses. From three crosses of γ het speltoids by speltoids he got seven het speltoids : six speltoids, 11 : 9, and 9 : 7. Together, these consistent results show that 120 normal and 104 mutant ovules produced mature plants. We may conclude that this represents approximately the proportion in which normal and mutant ovules are formed. If univalent loss is similar in ovules and pollen, our tetrad studies would give an expectation of 6.1 ovules with 20 chromosomes: 46.2 with 20 + Cd or Cts: 46.2 with 20 + C: 1.5 with 20 + C and an additional Cd or Cts chromosome that would not affect the phenotype in the presence of two C chromosomes. The cytologically "expected" proportion of "normal" and "mutant" ovules is therefore 47.7 : 52.3. In view of the variability in tetrad counts, the unknowns in the interpretation of micronuclei as indicators of gametic types formed in Series γ and the varying certation ratios shown in crosses with γ het speltoids as the pollen parent, no closer numerical agreement could be expected.

V. The Nature of the Speltoid Mutations

Series β het speltoids arise through loss of a C chromosome (in Sears' terminology they are "monosomic IX"). The missing chromosome carries factors essential to the expression of the "beardless normal" type of *T. vulgare*. As Watkins and Ellerton (41) have emphasized, the term "beardless" is a misnomer, but it is in general use. True beardless wheats occur but are not widely cultivated. There are two genetically different tip-awned types. "Tipped I" is the "beardless" type commonly grown in Europe and America and considered herein. The gene determining its type of tip-awn is on the C chromosome and is denoted B_1. Tipped 2, found chiefly in Asiatic wheats, is determined by a gene designated B_2. This symbolism is used to imply that the B genes are phylogenetically alleles, but now on different, probably homoeologous, chromosomes in these polyploids. The awn formula of European "beardless" wheats is $B_1B_1;b_2b_2$; that of the Asiatic is $b_1b_1;B_2B_2$. True beardless wheats are $B_1B_1;B_2B_2$, and $b_1b_1;b_2b_2$ is fully bearded. The gene or gene complex k (Watkins (40)) which determines "normal" *T. vulgare* glume shape and thickness is also on the C chromosome 27 or more units from the B locus. The C chromosome may therefore be symbolized as (B_1— 27 + — k) in ordinary "beardless" wheats and as (b_1— 27 + — k) in bearded varieties. Since Series γ het speltoids arise through loss of all or part of the long arm of the C chromosome, genes B_1 and k must be located in it. This long arm is marked by a secondary constriction about three-eighths

along its length. Chromosome configurations in het speltoids lacking part of the long arm, Fig. 12*a*, *b*, demonstrate that the deficiency, and therefore the segment B_1—k, is interstitial in the distal segment.

Bearded is definitely recessive, though to varying degrees in different crosses. The glume characters are intermediate in the heterozygote and opinions have differed whether speltoid ("K" according to Watkins) should be considered recessive or dominant. Nilsson-Leissner (26) pointed out that while the speltoid mutation as a whole may be considered recessive, in detail only beards are fully recessive. The head-type is nearer dominant and the glumes are intermediate. The "complex" mutations, he said, revealed the inadequacy of the dominance concepts then current. Lindhard (16) pointed out that those concepts derived from a too literal acceptance of the presence and absence hypothesis.

The genetic evidence makes it highly probable that a deficiency is involved in Series α as in γ—*contra* Uchikawa (36) and others who class α speltoids as gene mutations. Selfed het speltoids that have arisen at one step from the normal, i.e. by "total mutation", always give ratios that deviate in some degree from 1 : 2 : 1 towards 1 : 1 : 0, thus indicating reduced functioning of the mutant pollen. Nilsson-Ehle (25) showed further that in the "total-mutations" of any Series the interstitial region between the beard and glume factors must in some way be affected for no crossing over takes place between them, though it does in bearded speltoids that have originated by segregation following the crossing of part-mutants, i.e., beardless speltoids and bearded normals. He found 27 to 36% crossing over in studies of part-mutants, which is similar to the 28 to 39% found by Watkins in intervarietal and interspecific crosses which involved the B_1 and k loci. The ratios from selfed part-mutants do not usually deviate appreciably from 1 : 2 : 1. These data are all explicable on the assumption, adumbrated initially in 1921 by Nilsson-Ehle, that the total-mutation, whatever its Series, involves loss of not less than the B_1—27 + —k region of the C chromosomes. The extent of the loss—a microscopically indeterminable or doubtfully determinable segment in α, a readily determinable segment which may be as large as the entire longer arm in γ and loss of the entire chromosome in β—evidently determines in the strains here considered the degree to which chromosome behavior is affected in meiosis and also the extent to which mutant pollen is handicapped in competition with the normal in het speltoids.

Uchikawa's data on the segregation of F_1 hybrids between α and γ speltoids indicate Cts pollen is very little handicapped when in competition with C^{α}. Such F_1 speltoids having one α and one γ C chromosome (C^{α} Cts in this case) give only speltoid progeny but they are of three chromosome types, C^{α} C^{α}, C^{α} Cts, and CtsCts, in the ratio 1 : 2 : 0.6. In contrast, Cts pollen is severely handicapped when in competition with normal (C) pollen, for selfing CCts het speltoids gives a 1 : 1 : 0.05 ratio of CC, CCts, and CtsCts progeny.

In 1914, before it was known that *T. vulgare* is a polyploid species, Nilsson-Ehle conceived the normal phenotype as resulting from a balance of factors

tending to cause development on the one hand towards *T. spelta* in glume and spike characteristics and on the other towards *T. compactum*. Winge in 1924 fitted this concept to the polyploid status of these three species—see Huskins (11). The concept of Philiptschenko (29, 30) was superficially similar. He considered that a gene C thickens and shortens the ear while S produces lengthening: *T. compactum* is CCss, *T. spelta* ccSS, and *T. vulgare* ccss. The genes on his concept work as inhibiting factors affecting various parts of the developmental process.

The present cytogenetic data from speltoids and compactoids can most simply be fitted by the concept that factors B_1 and k are inhibitors or suppressors which in normal wheat counteract the effect of awn-producing and glume-thickening genes located on chromosomes other than C.*

If a bearded spelt-like form is considered the original wild type from which *T. vulgare* has been derived by mutation, then Watkins' factors k and B_1 are mutations of K and b_1 which produce the cultivated glume and tip-awn type. To use this formal genetic concept and yet recognize that deficiency of k, as in mutant speltoids, gives a phenotype similar to that produced by K and that the absence of B_1 gives approximately the same effect as the presence of b_1 it is only necessary to call K and b_1 "amorphs" (Muller (22)). Sears (32) has shown that b is almost though not quite an amorph since the nullisomic beardless speltoid, $--$; B_2B_2, has slightly longer awns than the normal, which is b_1b_1; B_2B_2, in his variety Chinese Spring. The polyploid nature and probable hybrid ancestry of *T. vulgare* help to make the inhibitor concept more acceptable than it might be in a diploid, but it must of course be recognized for the present as a formal genetic, not a physiological, interpretation.

In the present analyses of speltoids there is no way of determining where the "positive" speltoid glume and beard factors are located or whether they are single factors that in the ancestral diploid form, or forms, were true alleles of the present day inhibitors. Alternatively the beard and speltoid glume characters could be the expression of many genes, that is, of the "genetic background" of *T. vulgare*. Watkins' evidence indicates that different alleles distinguish various species and his symbolism implies that the K alleles are, through polyploidy, on homoeologous chromosomes. His formulae are:

T. vulgare	kk; KK; K^dK^d
T. spelta	K^sK^s; KK; K^dK^d
Speltoid	KK; KK; K^dK^d

This speltoid is not a mutant but the type that segregates from crosses of *T. vulgare* with *T. turgidum*. The three pairs of group symbols represent, of course, parts of three homoeologous pairs of chromosomes, present on account of the hexaploidy. K^s is the spelta gene, K the speltoid, and K^d, which may

* *McFadden and Sears (J. Heredity, 37 : 81) believe that the T. vulgare inhibitors of spelta characteristics may have been acquired by transfer of a block of genes from a Lake Dweller wheat into T. spelta.*

be identical with K^s, the gene producing the somewhat speltoid-like glumes of *T. dicoccum*. Speltoids involving deficiency would on this symbolism be – –; KK; K^dK^d. Those compactoids which involve the presence of an extra pair of C chromosomes would be kk; kk; KK; K^dK^d, while those that have a pair of isomorphic chromosomes, each constituted of two major arms of C, are kk kk; KK; K^dK^d.

Winge (42) assumed that a specific chromosome, B, carries the beard and speltoid glume factors. We can find no evidence for this nor can Sears (personal communication) though he now has trisomes for most of the 21 chromosomes. Many chromosomes seem to have an effect in producing or lengthening beards and in hardening the glumes. Polysomics II and XX have fairly stiff glumes, I and XVII have glumes less tough than normal, XV has shorter glumes (as has the speltoid) VI, X and XIX have broader, and probably tougher glumes, while in V and XVIII they are larger. Sears' evidence, then, favors the assumption that the speltoid phenotype is due to many genes—perhaps it is nearer the original wild type—and that the beardless cultivated type is determined predominantly by the two genes B_1 and k in European or B_2 and k in Asiatic wheats. Once such inhibitors arose they would naturally be selected by man because they produce a phenotype highly desirable agriculturally and would soon come to be part of the normal genotype of cultivated wheats.

It is an open question whether the speltoid mutation occurs oftener than other mutations in wheat, or is merely picked out more often on account of its striking characteristics. If the latter, it would be logical to assume that chance irregularities of mitosis and meiosis, affecting at random any chromosome, are responsible for the occurrence of β het speltoids. Those γ het speltoids which have lost the entire long arm of C could likewise be due to chance irregularities. Telokinetic chromosomes arise through misdivision of the kinomere in unpaired chromosomes, as do the isochromosomes that produce Type I subcompactoids. Unpaired chromosomes occur frequently in some varieties of wheat newly developed by hybridization (Hollingshead (7), Love (20, 21)), as do speltoids. It seems necessary, however, to assume some more specific and recurring abnormality as responsible for the interstitial deficiencies of α and most γ het speltoids. One obvious possibility is homoeologous pairing of the type BC postulated by Winge (42), but followed by double crossing over and hence substitution for the sector including B_1—k of one lacking it, instead of by his whole chromosome substitution. It is not necessary to assume that B carries speltoid factors, only that it lacks genes B_1 and k. The cytological evidence for homoeologous pairing of C after it has mutated (Winge and Huskins) is now known to be in large part faulty and at best slight. It is not, however, negligible and the evidence which Uchikawa (36) presents as disproving it actually favors the interpretation that when a normal C chromosome lacks a homologue it is likely to pair with some other chromosome, presumably a homoeologue. In β het speltoids he finds 0.31% trivalent formation, whereas in all other segregates of all Series,

the range is 0.08 to 0.2%. Multiple chromosome complexes are fairly common in "haploids" of the hexaploid wheats, but they occur also in true haploids of the diploid *T. monococcum* (Kihara and Katayama (15)) and in any case cannot be considered unequivocal evidence of homoeologous pairing, as interchange of segments also can produce them. Ellerton (4) observed chromosome catenation in the F_1 of *T. sphaerococcum* Perc. × *T. vulgare* and explained the high proportion of het speltoids found in F_2 as due to factors K_2 and k_1 being on the chromosomes involved in the reciprocal translocations responsible for the catenation. This is probably the simplest explanation for the high frequency with which het speltoids arise in many intervarietal hybrids of *T. vulgare*. His assumption that reciprocal translocations may account for the differences in frequency with which pure lines of *T. vulgare* produce het speltoid mutants would appear, however, to be valid only if he implies also duplication as a result of segregation after interchange, in addition to that present initially through polyploidy. The occurrence of true breeding hybrids between polyploids and of "Shift" (3) gives genetic evidence of homoeologous pairing. Watkins (40) interprets the lack of segregation for the K and K^d factors in the cross *T. turgidum* and *T. dicoccum* in this way, but cytological confirmation is lacking.

A second possibility for which there is some cytological, and perhaps genetic, evidence is that the B_1—k deficiency arises through crossing over in a duplicated region on C. There is evidence for duplication in the occurrence of occasional foldback pairing of C when it is univalent (Kerr, 13). Watkins (40) has evidence that the k gene is associated with something that affects pollen functioning. He suggests that this is a deficiency which is compensated for by a duplication on a member of a different genome. It seems that duplication on C and deficiency elsewhere would give the same result. If there is a duplication on C it is possible, though not essential to this interpretation of the origin of the B_1—k deficiency, that it is of the B_1 and k loci themselves, or of one of them. That region of C would then be similar to, but of greater extent than, the Bar locus in *Drosophila*, and deficiency would result from "unequal crossing over". Duplications of B_1, or k would, like the original mutation, be favored by human selection if they enhanced the inhibitory effect on awns and spelta-type glumes. Any possibly deleterious effect of duplication on pollen functioning would be less in a polyploid than a diploid and after a single generation, wheat being self-fertilized, there would be some plants homozygous for the duplication and in them there would be no competition with the original normal pollen.

The possibilities that may be envisaged to account for the B_1—k deficiency are obviously too numerous for any one of them to be considered very seriously at the present stage. If they are borne in mind, evidence favoring or excluding them may be obtained. What is clear is that the speltoid mutation is not a gene mutation. Those speltoids that arise as segregates from crosses with *T. turgidum* may, of course, be differentiated by single loci rather than chromosome sectors, though Watkins (39) stresses that k and B_1 are

probably gene complexes, not single genes, and Nilsson-Ehle concluded that even the part-mutations involve more than one gene. Watkins (40) attempted to clarify part of that issue by crossing his speltoids with *T. spelta*, but the F_2 segregates were too difficult to classify. It is clear also that speltoids are not, characteristically at least, due to whole chromosome substitutions as originally thought by Winge and Huskins. Finally, it is clear that they are not simple (Mendelian) segregates from crosses between *T. vulgare* and *T. spelta*, though forms similar to mutant speltoids may arise in this way, and hybridization, even between varieties, of wheat does result in irregularities of meiosis, some of which may produce the speltoid mutation.

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EXPLANATION OF PLATES

PLATE I

FIG. 23. *Strain β 1. Normal and het speltoid heads.*
FIG. 24. *Strain β 2. Normal, monosomic C (C–) het speltoid, and sterile nullisomic C (––) speltoid.*
FIG. 25. *Strain β 3. Normal-het speltoid chimera.*
FIG. 26. *Strain β 3. Normal, C– het speltoid, and anomalous fertile bearded––speltoid which has an extra non-C chromosome and two fragments.*

PLATE II

FIG. 27. *Strain β 4. Normal, C– het speltoid, and sterile dwarf––speltoid.*
FIG. 28. *Strain γ 3. Normal and CC– deficient (CCd) het speltoid.*
FIG. 29. *Strain γ 1. Normal, CCd het speltoid, C– het speltoid, and CdCd speltoid.*
FIG. 30. *Strain γ 4. Normal, and CC-telokinetic short arm (CCts) het speltoid.*

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PLATE II

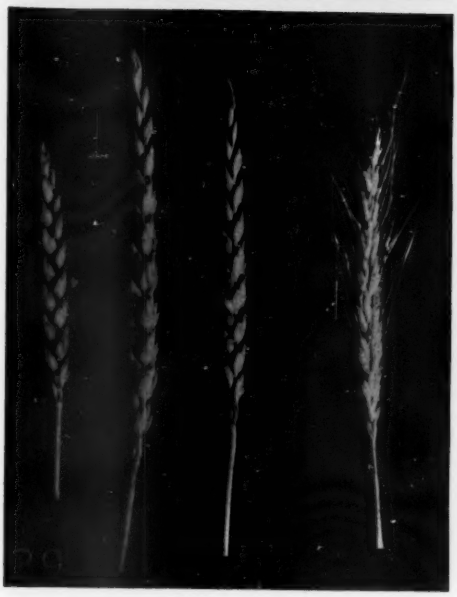
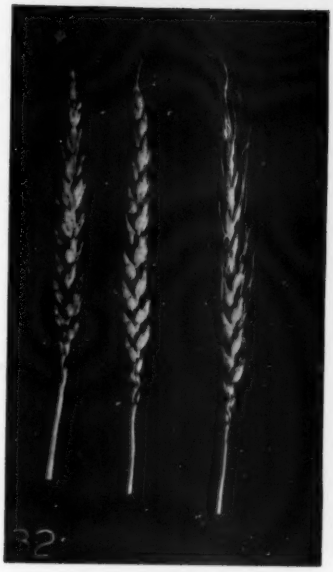
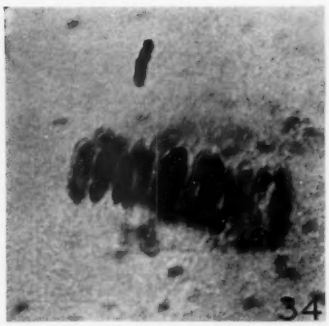


PLATE III

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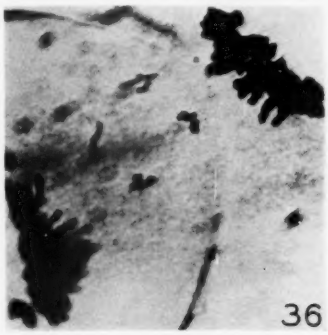
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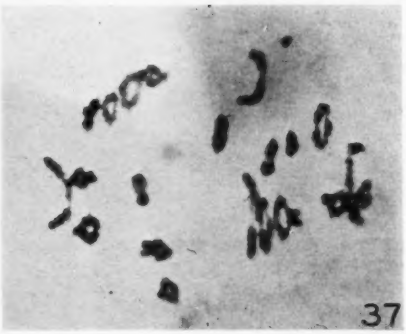
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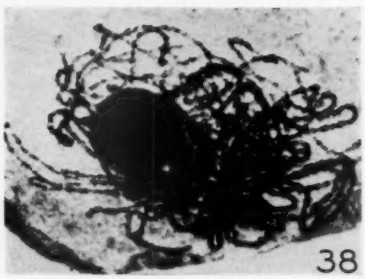
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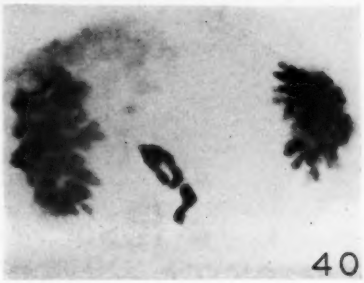
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PLATE III

FIG. 31. *Strain γ 2. Normal, CCd het speltoid, C– het speltoid, CdCd speltoid, Cd– speltoid, anomalous C– het speltoid with an extra non-C chromosome, anomalous – – speltoid (lacking C but having an extra non-C chromosome).*

FIG. 32. *Strain α 1. Normal, CC^{α} het speltoid and $C^{\alpha}C^{\alpha}$ speltoid.*

FIG. 33. *Strain α 2. Normal, normal-het speltoid chimera, CC^{α} het speltoid, and $C^{\alpha}C^{\alpha}$ beardless speltoid.*

PLATE IV

Photomicrographs of acetocarmine smear preparations of pollen mother cells.

FIG. 34. *Metaphase I, short-bearded het speltoid, strain β2. Note univalent "C" chromosome ("C_I") off the plate.*

FIG. 35. *Metaphase I (chromosomes scattered by pressure), het speltoid, strain γ2. The C and C-deficient ("Cd") chromosomes are unassociated.*

FIG. 36. *Anaphase I, het speltoid, strain γ1. The unassociated C and Cd have "split" after the bivalents have disjoined and their constituent chromatids are moving belatedly towards the poles.*

FIG. 37. *Metaphase I, het speltoid, strain γ2. C and Cd are associated forming a heteromorphic bivalent (CCd_{II}).*

FIG. 38. *Pachytene, het speltoid, strain γ2, interstitial deficiency demonstrated by unequal length of paired strands, presumably C and Cd, in a loop projecting from the main mass.*

FIG. 39. *Metaphase I, het speltoid, strain α 2, $20_{II} + CC^{\alpha}{}_{II}$ (very slight heteromorphism).*

FIG. 40. *Anaphase I, anomalous bearded speltoid ex γ2. A non-C trivalent has failed to be included in the daughter nuclei.*

FIG. 41. *Metaphase I showing heteromorphic bivalent from one head of a het speltoid-normal chimera.*

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